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(54) 【発明の名称】 ペプチドとその用途

(57) 【要約】

【課題】 本質的にスギ花粉アレルギーのT細胞エпитーブからなるペプチド及びそれに相同的なペプチドと、それらペプチドを有効成分として含んでなる免疫療法剤を提供する。

【解決手段】 スギ花粉アレルギーに特異的なイムノグロブリンE抗体に実質的に反応せず、<sup>3</sup>H-チミジンの取込みにより判定する方法で試験すると、陰性対照と比較して、スギ花粉アレルギーに特異的なT細胞を有意に活性化するペプチドと、そのペプチドを有効成分として含んでなる免疫療法剤を構成とする。

## 【特許請求の範囲】

【請求項1】 スギ花粉アレルゲンに特異的なイムノグロブリンE抗体に実質的に反応せず、<sup>1</sup>H-チミジンの取込みにより判定する方法で試験すると、陰性対照と比較して、スギ花粉アレルゲンに特異的なT細胞を有意に活性化するペプチド。

【請求項2】 配列表における配列番号1乃至7に示すアミノ酸配列のいずれかを含んでなる請求項1に記載のペプチド。

【請求項3】 配列表における配列番号8乃至13に示すいずれかのアミノ酸配列又はそのアミノ酸配列に相同的なアミノ酸配列を含んでなる請求項1又は2に記載のペプチド。

【請求項4】 請求項1に記載のペプチドにおける配列表の配列番号1に記載のアミノ酸配列。

【請求項5】 請求項1に記載のペプチドにおける配列表の配列番号2に記載のアミノ酸配列。

【請求項6】 請求項1に記載のペプチドにおける配列表の配列番号3に記載のアミノ酸配列。

【請求項7】 請求項1に記載のペプチドにおける配列表の配列番号4に記載のアミノ酸配列。

【請求項8】 請求項1に記載のペプチドにおける配列表の配列番号5に記載のアミノ酸配列。

【請求項9】 請求項1に記載のペプチドにおける配列表の配列番号6に記載のアミノ酸配列。

【請求項10】 請求項1に記載のペプチドにおける配列表の配列番号7に記載のアミノ酸配列。

【請求項11】 有効成分として請求項1乃至3に記載のペプチドを含んでなる免疫療法剤。

【請求項12】 有効成分として請求項1乃至3に記載のペプチドを0.01乃至100% (w/w) 含んでなる請求項11に記載の免疫療法剤。

【請求項13】 安定剤又は賦形剤として血清アルブミン、ゼラチン、マンニトール、マルトース及び/又はトレハロースを含む請求項11又は12に記載の免疫療法剤。

## 【発明の詳細な説明】

【0001】

【発明の属する技術分野】 この発明は、新規ペプチドとその用途、とりわけ、スギ花粉アレルゲンに特異的なT細胞を活性化するペプチドと、そのペプチドを有効成分として含んでなる免疫療法剤に関する。

【0002】

【従来の技術】 ここ十数年来、我国においては、春先になるとスギ花粉症による鼻炎や結膜炎を訴える人の数が増加し続けている。患者の数が多いことと、発症季がいろいろな行事が続く春先ということもあり、マスコミなどでも頻繁に取上げられ、今や、公衆衛生上無視できない問題の一つになっている。

【0003】 スギ花粉症はアレルギー症の一種であり、

その主因はスギ花粉中の抗原性物質、すなわち、スギ花粉アレルゲンであると云われている。大気中に飛散したスギ花粉がヒトの体内に侵入すると、スギ花粉アレルゲンに対するイムノグロブリンE抗体が産生する。この状態で次にスギ花粉が侵入すると、その花粉中のアレルゲンとこのイムノグロブリンE抗体が免疫反応を起し、アレルギー症状を呈することとなる。

【0004】 現在、スギ花粉中には、抗原性の相違する少なくとも二種類のアレルゲンの存在することが知られている。その一つは、ヤスエダらが「ジャーナル・オブ・アレルギー・アンド・クリニカル・イムノロジー」、第71巻、第1号、第77〜86頁(1983年)に報告しているアレルゲンであり、今日、これは「Cry j I」と呼称されている。もう一つは、タニアイら「エフ・イー・ビー・エス・レターズ」、第239巻、第2号、第329〜332頁(1988年)やサカグチら「アレルギー」、第45号、第309〜312頁(1990年)に報告されているアレルゲンであり、今日、これは「Cry j II」と呼称されている。スギ花粉中には、通常、Cry j IとCry j IIが約50:1乃至5:1の割合で存在し、花粉症患者から採取した血清の殆どがCry j IにもCry j IIにも反応すると云われている。澤谷らは、「アレルギー」、第42巻、第6号、第738〜747頁(1993年)において、Cry j IIが、皮内試験やRAS T試験すると、Cry j Iと同程度の抗原性を発揮すると報告している。

【0005】 このように、スギ花粉アレルゲンが既に幾つか単離され、その性質・性状もある程度解明されたことから、精製スギ花粉アレルゲンをヒトに投与して減感作することにより、スギ花粉症を治療・予防できる見通しがついてきた。最近ではそのための減感作剤も幾つか考案されており、例えば、特開平1-156926号公報や特開平3-93730号公報には、N末端からのアミノ酸配列がAsp-Asn-Pro-Ile-Asp-Ser又はAla-Ile-Asn-Ile-Phe-Asnで表わされるスギ花粉アレルゲンに糖質を共有結合せしめ、生成した複合体を減感作剤としてヒトに投与する提案が為されている。しかしながら、アレルギー症の診断や減感作療法には、通常、高純度のアレルゲンが大量に必要とされるところ、スギ花粉中のアレルゲンは僅少であるうえに安定性が低く、スギ花粉症の診断剤や減感作剤をスギ花粉だけで賄おうとすると、多大の困難が伴うと予想される。

【0006】 このようなことから、最近のアレルギー疾患の治療・予防においては、これまでのように、患者にアレルゲン全体を投与するのではなく、アレルゲンにおけるT細胞が特異的に認識する最小領域、すなわち、本質的にT細胞エпитープからなる低分子のペプチドを投与する免疫療法が注目を浴びつつある。

【0007】一般に、アレルゲンは、マクロファージなどの抗原提示細胞に取込まれると、そこで消化され、消化断片が免疫提示細胞表層のHLA蛋白質に結合し、抗原提示されることとなる。抗原提示される断片は、HLA蛋白質に対する親和性などにより、アレルゲンにおける一部の特定領域に限られ、斯かる領域のうち、T細胞が特異的に認識する領域は、通常、「T細胞エпитープ」と呼称される。本質的にT細胞エпитープからなるペプチドを投与する免疫療法には、

(i) ペプチドがB細胞エпитープを欠いている、すなわち、アレルゲンに特異的なイムノグロブリンE抗体が反応しないので、従来の粗製又は精製アレルゲンで頻発していたアナフィラキシーなどの副作用が起り得ない。

(ii) 少量からスタートし、有効投与量に達するまでの期間が、従来の減感作剤に比較して、大幅に短縮できる。などの利点がある。

【0008】目下、種々のアレルゲンのT細胞エпитープが精力的に解析されているが、T細胞エпитープの解析には、通常、アレルゲンの全アミノ酸配列が必須となり、少なくともスギ花粉アレルゲンに関するかぎり、T細胞エпитープは実質的に解明されるに到っていないというのが実状である。

【0009】

【発明が解決しようとする課題】斯かる状況に鑑み、この発明の第一の課題は、本質的にスギ花粉アレルゲンのT細胞エпитープからなるペプチド及びそれに相同的なペプチドを提供することにある。

【0010】この発明の第二の課題は、有効成分として上記ペプチドを含んでなる免疫療法剤を提供することにある。

【0011】

【課題を解決するための手段】この発明は、前記第一の課題を、スギ花粉アレルゲンに特異的なイムノグロブリンE抗体に実質的に反応せず、<sup>3</sup>H-チミジンの取込みにより判定する方法で試験すると、陰性対照と比較して、スギ花粉アレルゲンに特異的なT細胞を有意に活性化するペプチドにより解決するものである。

【0012】この発明は、前記第二の課題を、有効成分として斯かるペプチドを含んでなる免疫療法剤により解決するものである。

【0013】

【発明の実施の形態】この発明のペプチドは、スギ花粉アレルゲンに特異的なイムノグロブリンE抗体に実質的に反応しないので、ヒトを含む哺乳類一般に投与すると、実質的にアナフィラキシーを引起すことなく、スギ花粉アレルゲンに特異的なT細胞を活性化する。

【0014】有効成分として斯かるペプチドを含んでなるこの発明の免疫療法剤は、ヒトを含む哺乳類一般に投与すると、実質的にアナフィラキシーを引起すことな

く、スギ花粉症に対して顕著な治療・予防効果を発揮する。

【0015】以下、実験例、実施例等によりこの発明を説明するに、この発明は、本質的にスギ花粉アレルゲンのT細胞エпитープからなるペプチドの発見に基づくものである。

【0016】本発明者らは、長年に亙るスギ花粉アレルゲンに係わる研究の一成果として、昨年、スギ花粉アレルゲンの主たる1成分が配列表における配列番号14に示すアミノ酸配列を有することを突止め、特願平5-344596号明細書に開示した。一方、国際特許公開第93/01213号明細書には、スギ花粉アレルゲンの別の1成分が、配列表における配列番号15に示すアミノ酸配列を有すると開示されており、本発明者らも、平成6年4月14乃至16日に熊本県熊本市で開催された「第6回日本アレルギー学会春期臨床大会」において、同じアミノ酸配列を発表している。

【0017】そこで、本発明者が、スギ花粉アレルゲンのT細胞エпитープを解明すべく、これら配列番号14及び15に示すアミノ酸配列に基づき、それらアミノ酸配列における連続する11、14又は17個のアミノ酸残基からなる、180余種の互いに相違するアミノ酸配列のペプチドを合成し、スギ花粉アレルゲンに特異的なイムノグロブリンE抗体に対する反応性と、スギ花粉アレルゲンに特異的なT細胞に対する活性化作用につき試験した。その結果、配列表における配列番号8乃至13に示すアミノ酸配列を有するペプチドは、スギ花粉アレルゲンに特異的なイムノグロブリンE抗体に実質的に反応せず、また、<sup>3</sup>H-チミジンの取込みにより判定する方法で試験すると、陰性対照と比較して、スギ花粉アレルゲンに特異的なT細胞を有意に活性化することが明らかとなった。このことは、それら配列番号8乃至13に示すアミノ酸配列を有するペプチドが本質的にスギ花粉アレルゲンのT細胞エпитープからなるものであることを示唆している。また、その配列番号8乃至13に示すアミノ酸配列をさらに解析したところ、配列表の配列番号1乃至7に示すアミノ酸配列は、T細胞が配列番号8乃至13に示すアミノ酸配列のペプチドを認識するために不可欠の配列であることが判明した。

【0018】次の実験例1及び2では、これら事実を解明するに到った一連の実験について説明する。

【0019】

【実験例1 ペプチド及びスギ花粉アレルゲンの調製】

【0020】

【実験例1-1 ペプチドの調製】前述のとおり、これまで、スギ花粉には、性質・性状の相違する、少なくとも2種類のアレルゲンの存在することが知られている。これらスギ花粉アレルゲンの成熟蛋白質は、組換えDNA技術により、配列表における配列番号14又は15に示すアミノ酸配列を有することが明らかにされており、

現に、スギ花粉からは、配列番号14に示すアミノ酸配列における第46乃至433番目又は第51乃至433番目に相当するアミノ酸配列のスギ花粉アレルゲン（以下、「アレルゲンA」と云う。）と、配列番号15に示すアミノ酸配列における第1乃至353番目のアミノ酸配列を有するスギ花粉アレルゲン（以下、「アレルゲンB」と云う。）が単離されている。なお、アレルゲンAをコードする遺伝子においては、未だ、シグナルペプチドが確定されていないので、配列番号14においては、暫定的に、cDNAの塩基配列から解読したアミノ酸配列におけるN末端側の最初のアミノ酸残基に符号「1」を付している。

【0021】本実験例では、配列表における配列番号14に示すアミノ酸配列については、その第46乃至433番目の領域に互り、アミノ酸残基を10個ずつ重複させながら、11又は14個のアミノ酸残基からなる、95種類の相違するアミノ酸配列のペプチド（試料A-1乃至A-95）を化学合成する一方、配列番号15に示すアミノ酸配列については、その第1乃至353番目の領域に互り、同じく、アミノ酸残基を10個ずつ重複させながら、14個のアミノ酸残基からなる、86種類の相違するアミノ酸配列のペプチド（試料B-1乃至B-86）を化学合成し、この発明のペプチドを検索するための後記実験例2に供した。

【0022】すなわち、常法にしたがって、11又は14個のアミノ酸残基からなり、後記表1乃至6に示すアミノ酸配列を有する181種類のペプチドをケンブリッジ・リサーチ・バイオケミカルズ製ペプチド合成キット「マルチピン」を使用する固相法により合成し、合成後、その一部をとり、パーキン・エルマー製ペプチドシーケンサー「470A型」により分析して所期のアミノ酸配列を有していることを確認した。

【0023】

【実験例1-2 スギ花粉アレルゲンの調製】秋田県産ウラスギの雄花から採取した花粉1重量部を約16重量部の0.125M炭酸水素ナトリウム水溶液（pH8.2）に浸漬し、穏やかに攪拌しながら、4℃で1時間抽出した。抽出物を遠心分離し、残渣を上記と同様に再度抽出し、得られた上清と初回の上清をブールし、これにセタブロンを0.1%（w/v）になるように加え、緩やかに攪拌しながら、4℃で1時間静置して多糖類を沈澱させ、遠心分離後、上清に硫酸アンモニウムを80%飽和になるように加え、4℃で一昼夜静置して塩析した。

【0024】塩析物における沈澱部を採取し、これを50mMトリス-塩酸緩衝液（pH7.8）に対して10時間透析し、濾過後、予め50mMトリス-塩酸緩衝液（pH7.8）で平衡化させておいたDEAE-セファデックスカラムに負荷し、カラムに新鮮な同一緩衝液を

通液して蛋白質成分を含む画分を溶出させた。この画分を採取し、酢酸を加えてpH5.0に調整後、予め10mM酢酸緩衝液（pH5.0）で平衡化させておいたCM-セファデックスカラムに負荷し、カラムを10mM酢酸緩衝液（pH5.0）で洗浄後、カラムに0.3M塩化ナトリウムを含む0.1M酢酸緩衝液（pH7.0）を通液し、蛋白質成分を含む画分を採取した。

【0025】次に、この画分に予め10mM酢酸緩衝液（pH5.0）で平衡化させておいたMono Sカラムに負荷し、カラムを10mM酢酸緩衝液（pH5.0）で洗浄後、0Mから0.5Mに上昇する塩化ナトリウムの濃度勾配下、カラムに10mM酢酸緩衝液（pH7.0）を通液したところ、0.1乃至0.3付近の塩化ナトリウム濃度でアレルゲンBが、また、0.4M付近の塩化ナトリウム濃度でアレルゲンAが溶出した。アレルゲンA又はBを含む画分を別々に採取し、適宜濃縮後、凍結乾燥して次の実験例2に供した。収量は、原料スギ花粉固形分当たり、アレルゲンAで約0.01%、アレルゲンBで約0.02%であった。

【0026】

【実験例2 スギ花粉アレルゲンのT細胞エビトープを含むペプチドの検索】

【0027】

【実験例2-1 スギ花粉アレルゲンに特異的なT細胞の活性化】フィコール・ハイバック比重遠心法により、花粉症患者のヘパリン加末梢血からスギ花粉アレルゲンに特異的なT細胞を含む単核細胞群を分離した。この単核細胞群を5%（v/v）AB血清を補足したRPMI 1640培地（pH7.0）に浮遊させ、96ウェルマイクロプレート上に5×10<sup>5</sup>個/ウェルずつ分注し、実験例1-1及び1-2で調製したペプチド又はスギ花粉アレルゲンを1μg/ウェル加え、新鮮な同一培地で200μl/ウェルとした後、5%CO<sub>2</sub>培養器中、37℃で2日間インキュベートした。その後、<sup>3</sup>H-チミジンを1.0μCi/ウェルずつ加え、同一条件下でさらに16時間インキュベートした後、シンチレーションカウンタを使用する公知の方法により、単核細胞群における<sup>3</sup>H-チミジンの取込み量を測定した。同時に、ペプチドもスギ花粉アレルゲンも含まない系を設け、上記と同様に処置して陰性対照とした。

【0028】スギ花粉アレルゲンに特異的なT細胞に対する活性化作用の有無は、同T細胞を含む単核細胞群における<sup>3</sup>H-チミジンの取込み量（cpm）に基づき判定し、取込み量が陰性対照の略2倍以上に達した系を「陽性」、達しなかった系を「陰性」とした。結果を表1乃至6に示す。

【0029】

【表1】

試料	位置	アミノ酸配列	T細胞の活性化	ヒトイムノグロブリンE抗体 に対する反応性 (M492)
A-1	46-59	Arg-Lys-Val-Glu-His-Ser-Arg-His-Asp-Ala-Ile-Asn-Ile-Phe	陰性	-
A-2	51-64	Ser-Arg-His-Asp-Ala-Ile-Asn-Ile-Phe-Asn-Val-Glu-Lys-Tyr	陰性	-
A-3	55-68	Ala-Ile-Asn-Ile-Phe-Asn-Val-Glu-Lys-Tyr-Gly-Ala-Val-Gly	陰性	-
A-4	59-72	Phe-Asn-Val-Glu-Lys-Tyr-Gly-Ala-Val-Gly-Asp-Gly-Lys-His	陰性	-
A-5	63-76	Lys-Tyr-Gly-Ala-Val-Gly-Asp-Gly-Lys-His-Asp-Cys-Thr-Glu	陰性	-
A-6	67-80	Val-Gly-Asp-Gly-Lys-His-Asp-Cys-Thr-Glu-Ala-Phe-Ser-Thr	陰性	-
A-7	71-84	Lys-His-Asp-Cys-Thr-Glu-Ala-Phe-Ser-Thr-Ala-Trp-Gln-Ala	陰性	-
A-8	75-88	Thr-Glu-Ala-Phe-Ser-Thr-Ala-Trp-Gln-Ala-Ala-Cys-Lys-Lys	陰性	-
A-9	79-92	Ser-Thr-Ala-Trp-Gln-Ala-Ala-Cys-Lys-Lys-Pro-Ser-Ala-Met	陰性	-
A-10	83-96	Gln-Ala-Ala-Cys-Lys-Lys-Pro-Ser-Ala-Met-Leu-Leu-Val-Pro	陰性	-
A-11	87-100	Lys-Lys-Pro-Ser-Ala-Met-Leu-Leu-Val-Pro-Gly-Asn-Lys-Lys	陰性	-
A-12	91-104	Ala-Met-Leu-Leu-Val-Pro-Gly-Asn-Lys-Lys-Phe-Val-Val-Asn	陰性	-
A-13	95-108	Val-Pro-Gly-Asn-Lys-Lys-Phe-Val-Val-Asn-Asn-Leu-Phe-Phe	陰性	-
A-14	99-112	Lys-Lys-Phe-Val-Val-Asn-Asn-Leu-Phe-Phe-Asn-Gly-Pro-Cys	陰性	-
A-15	103-116	Val-Asn-Asn-Leu-Phe-Phe-Asn-Gly-Pro-Cys-Gln-Pro-His-Phe	陰性	-
A-16	107-120	Phe-Phe-Asn-Gly-Pro-Cys-Gln-Pro-His-Phe-Thr-Phe-Lys-Val	陰性	-
A-17	111-124	Pro-Cys-Gln-Pro-His-Phe-Thr-Phe-Lys-Val-Asp-Gly-Ile-Ile	陰性	-
A-18	115-128	His-Phe-Thr-Phe-Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln	陰性	-
A-19	119-132	Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser	陰性	-
A-20	123-136	Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser-Trp-Lys-Asn-Asn	陽性	-
A-21	127-140	Tyr-Gln-Asn-Pro-Ala-Ser-Trp-Lys-Asn-Asn-Arg-Ile-Trp-Leu	陰性	-
A-22	131-144	Ala-Ser-Trp-Lys-Asn-Asn-Arg-Ile-Trp-Leu-Gln-Phe-Ala-Lys	陰性	-
A-23	135-148	Asn-Asn-Arg-Ile-Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe	陽性	-
A-24	139-152	Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly	陰性	-
A-25	143-156	Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly-Lys-Gly-Val-Ile	陰性	-
A-26	147-160	Gly-Phe-Thr-Leu-Met-Gly-Lys-Gly-Val-Ile-Asp-Gly-Gln-Gly	陰性	-
A-27	151-164	Met-Gly-Lys-Gly-Val-Ile-Asp-Gly-Gln-Gly-Lys-Gln-Trp-Trp	陰性	-
A-28	155-168	Val-Ile-Asp-Gly-Gln-Gly-Lys-Gln-Trp-Trp-Ala-Gly-Gln-Cys	陰性	-
A-29	159-172	Gln-Gly-Lys-Gln-Trp-Trp-Ala-Gly-Gln-Cys-Lys-Trp-Val-Asn	陰性	-
A-30	163-176	Trp-Trp-Ala-Gly-Gln-Cys-Lys-Trp-Val-Asn-Gly-Arg-Glu-Ile	陰性	-
A-31	167-180	Gln-Cys-Lys-Trp-Val-Asn-Gly-Arg-Glu-Ile-Cys-Asn-Asp-Arg	陰性	-

【表2】

A-32	171-184	Val-Asn-Gly-Arg-Glu-Ile-Cys-Asn-Asp-Arg-Asp-Arg-Pro-Thr	性	-
A-33	175-188	Glu-Ile-Cys-Asn-Asp-Arg-Asp-Arg-Pro-Thr-Ala-Ile-Lys-Phe	陰	-
A-34	179-192	Asp-Arg-Asp-Arg-Pro-Thr-Ala-Ile-Lys-Phe-Asp-Phe-Ser-Thr	陰	-
A-35	183-196	Pro-Thr-Ala-Ile-Lys-Phe-Asp-Phe-Ser-Thr-Gly-Leu-Ile-Ile	陰	-
A-36	187-200	Lys-Phe-Asp-Phe-Ser-Thr-Gly-Leu-Ile-Ile-Gln-Gly-Leu-Lys	陰	-
A-37	191-204	Ser-Thr-Gly-Leu-Ile-Ile-Gln-Gly-Leu-Lys-Leu-Met-Asn-Ser	陰	-
A-38	195-208	Ile-Ile-Gln-Gly-Leu-Lys-Leu-Met-Asn-Ser-Pro-Glu-Phe-His	陰	-
A-39	199-212	Leu-Lys-Leu-Met-Asn-Ser-Pro-Glu-Phe-His-Leu-Val-Phe-Gly	陰	-
A-40	203-216	Asn-Ser-Pro-Glu-Phe-His-Leu-Val-Phe-Gly-Asn-Cys-Glu-Gly	陰	-
A-41	207-220	Phe-His-Leu-Val-Phe-Gly-Asn-Cys-Glu-Gly-Val-Lys-Ile-Ile	陰	-
A-42	211-224	Phe-Gly-Asn-Cys-Glu-Gly-Val-Lys-Ile-Ile-Gly-Ile-Ser-Ile	陰	-
A-43	215-228	Glu-Gly-Val-Lys-Ile-Ile-Gly-Ile-Ser-Ile-Thr-Ala-Pro-Arg	陰	-
A-44	219-232	Ile-Ile-Gly-Ile-Ser-Ile-Thr-Ala-Pro-Arg-Asp-Ser-Pro-Asn	陰	-
A-45	223-236	Ser-Ile-Thr-Ala-Pro-Arg-Asp-Ser-Pro-Asn-Thr-Asp-Gly-Ile	陰	-
A-46	227-240	Pro-Arg-Asp-Ser-Pro-Asn-Thr-Asp-Gly-Ile-Asp-Ile-Phe-Ala	陰	-
A-47	231-244	Pro-Asn-Thr-Asp-Gly-Ile-Asp-Ile-Phe-Ala-Ser-Lys-Asn-Phe	陰	-
A-48	235-248	Gly-Ile-Asp-Ile-Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys	陽	-
A-49	239-252	Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn-Thr-Ile-Gly	陽	-
A-50	243-256	Asn-Phe-His-Leu-Gln-Lys-Asn-Thr-Ile-Gly-Thr-Gly-Asp-Asp	陰	-
A-51	247-260	Gln-Lys-Asn-Thr-Ile-Gly-Thr-Gly-Asp-Asp-Cys-Val-Ala-Ile	陰	-
A-52	251-264	Ile-Gly-Thr-Gly-Asp-Asp-Cys-Val-Ala-Ile-Gly-Thr-Gly-Ser	陰	-
A-53	255-268	Asp-Asp-Cys-Val-Ala-Ile-Gly-Thr-Gly-Ser-Ser-Asn-Ile-Val	陰	-
A-54	259-272	Ala-Ile-Gly-Thr-Gly-Ser-Ser-Asn-Ile-Val-Ile-Glu-Asp-Leu	陰	-
A-55	263-276	Gly-Ser-Ser-Asn-Ile-Val-Ile-Glu-Asp-Leu-Ile-Cys-Gly-Pro	陰	-
A-56	267-280	Ile-Val-Ile-Glu-Asp-Leu-Ile-Cys-Gly-Pro-Gly-His-Gly-Ile	陰	-
A-57	271-284	Asp-Leu-Ile-Cys-Gly-Pro-Gly-His-Gly-Ile-Ser-Ile-Gly-Ser	陰	-
A-58	275-288	Gly-Pro-Gly-His-Gly-Ile-Ser-Ile-Gly-Ser-Leu-Gly-Arg-Glu	陰	-
A-59	279-292	Gly-Ile-Ser-Ile-Gly-Ser-Leu-Gly-Arg-Glu-Asn-Ser-Arg-Ala	陰	-
A-60	283-296	Gly-Ser-Leu-Gly-Arg-Glu-Asn-Ser-Arg-Ala-Glu-Val-Ser-Tyr	陰	-
A-61	287-300	Arg-Glu-Asn-Ser-Arg-Ala-Glu-Val-Ser-Tyr-Val-His-Val-Asn	陰	-
A-62	291-304	Arg-Ala-Glu-Val-Ser-Tyr-Val-His-Val-Asn-Gly-Ala-Lys-Phe	陰	-
A-63	295-308	Ser-Tyr-Val-His-Val-Asn-Gly-Ala-Lys-Phe-Ile-Asp-Thr-Gln	陰	-
A-64	299-312	Val-Asn-Gly-Ala-Lys-Phe-Ile-Asp-Thr-Gln-Asn-Gly-Leu-Arg	陰	-

【表3】

A-65	303-316	Lys-Phe-Ile-Asp-Thr-Gln-Asn-Gly-Leu-Arg-Ile-Lys-Thr-Trp	性	-
A-66	307-320	Thr-Gln-Asn-Gly-Leu-Arg-Ile-Lys-Thr-Trp-Gln-Gly-Gly-Ser	性	-
A-67	311-324	Leu-Arg-Ile-Lys-Thr-Trp-Gln-Gly-Gly-Ser-Gly-Met-Ala-Ser	性	-
A-68	315-328	Thr-Trp-Gln-Gly-Gly-Ser-Gly-Met-Ala-Ser-His-Ile-Ile-Tyr	性	-
A-69	219-332	Gly-Ser-Gly-Met-Ala-Ser-His-Ile-Ile-Tyr-Glu-Asn-Val-Glu	性	-
A-70	223-336	Ala-Ser-His-Ile-Ile-Tyr-Glu-Asn-Val-Glu-Met-Ile-Asn-Ser	性	-
A-71	227-340	Ile-Tyr-Glu-Asn-Val-Glu-Met-Ile-Asn-Ser-Glu-Asn-Pro-Ile	性	-
A-72	231-344	Val-Glu-Met-Ile-Asn-Ser-Glu-Asn-Pro-Ile-Leu-Ile-Asn-Gln	性	-
A-73	235-348	Asn-Ser-Glu-Asn-Pro-Ile-Leu-Ile-Asn-Gln-Phe-Tyr-Cys-Thr	性	-
A-74	239-352	Pro-Ile-Leu-Ile-Asn-Gln-Phe-Tyr-Cys-Thr-Ser-Ala-Ser-Ala	性	-
A-75	243-356	Asn-Gln-Phe-Tyr-Cys-Thr-Ser-Ala-Ser-Ala-Cys-Gln-Asn-Gln	性	-
A-76	347-360	Cys-Thr-Ser-Ala-Ser-Ala-Cys-Gln-Asn-Gln-Arg-Ser-Ala-Val	性	-
A-77	351-364	Ser-Ala-Cys-Gln-Asn-Gln-Arg-Ser-Ala-Val-Gln-Ile-Gln-Asp	性	-
A-78	355-368	Asn-Gln-Arg-Ser-Ala-Val-Gln-Ile-Gln-Asp-Val-Thr-Tyr-Lys	性	-
A-79	359-372	Ala-Val-Gln-Ile-Gln-Asp-Val-Thr-Tyr-Lys-Asn-Ile-Arg-Gly	性	-
A-80	363-376	Gln-Asp-Val-Thr-Tyr-Lys-Asn-Ile-Arg-Gly-Thr-Ser-Ala-Thr	性	-
A-81	367-380	Tyr-Lys-Asn-Ile-Arg-Gly-Thr-Ser-Ala-Thr-Ala-Ala-Ile	性	-
A-82	371-384	Arg-Gly-Thr-Ser-Ala-Thr-Ala-Ala-Ile-Gln-Leu-Lys-Cys	性	-
A-83	375-388	Ala-Thr-Ala-Ala-Ala-Ile-Gln-Leu-Lys-Cys-Ser-Asp-Ser-Met	性	-
A-84	379-392	Ala-Ile-Gln-Leu-Lys-Cys-Ser-Asp-Ser-Met-Pro-Cys-Lys-Asp	性	-
A-85	383-396	Lys-Cys-Ser-Asp-Ser-Met-Pro-Cys-Lys-Asp-Ile-Lys-Leu-Ser	性	-
A-86	387-400	Ser-Met-Pro-Cys-Lys-Asp-Ile-Lys-Leu-Ser-Asp-Ile-Ser-Leu	性	-
A-87	391-404	Lys-Asp-Ile-Lys-Leu-Ser-Asp-Ile-Ser-Leu-Lys-Leu-Thr-Ser	性	-
A-88	395-408	Ser-Leu-Lys-Leu-Thr-Ser-Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala	性	-
A-89	399-412	Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn-Asp-Ala-Asn	性	-
A-90	403-416	Ile-Ala-Ser-Cys-Leu-Asn-Asp-Ala-Asn-Gly-Tyr-Phe-Ser	陽	-
A-91	407-420	Leu-Asn-Asp-Ala-Asn-Gly-Tyr-Phe-Ser-Gly-His-Val-Ile	陰	-
A-92	411-424	Ala-Asn-Gly-Tyr-Phe-Ser-Gly-His-Val-Ile-Pro-Ala-Cys-Lys	陰	-
A-93	415-428	Phe-Ser-Gly-His-Val-Ile-Pro-Ala-Cys-Lys-Asn-Leu-Ser-Pro	陰	-
A-94	419-432	Val-Ile-Pro-Ala-Cys-Lys-Asn-Leu-Ser-Pro-Ser	陰	-
A-95	423-433	Asp-Asn-Pro-Ile-Cys-Lys-Asn-Leu-Ser-Pro-Ser	陰	-
B-1	1-14	Asp-Ser-Cys-Trp-Arg-Gly-Asp-Ser-Asn-Trp-Ala-Gln-Asn-Arg	性	-
B-2	5-18		性	-

【表4】

B-3	9-22	Arg-Gly-Asp-Ser-Asn-Trp-Ala-Gln-Asn-Arg-Met-Lys-Leu-Ala	性	-
B-4	13-26	Asn-Trp-Ala-Gln-Asn-Arg-Met-Lys-Leu-Ala-Asp-Cys-Ala-Val	陰	-
B-5	17-30	Asn-Arg-Met-Lys-Leu-Ala-Asp-Cys-Ala-Val-Gly-Phe-Gly-Ser	性	-
B-6	21-34	Leu-Ala-Asp-Cys-Ala-Val-Gly-Phe-Gly-Ser-Ser-Thr-Met-Gly	陰	-
B-7	25-38	Ala-Val-Gly-Phe-Gly-Ser-Ser-Thr-Met-Gly-Gly-Lys-Gly-Gly	性	-
B-8	29-42	Gly-Ser-Ser-Thr-Met-Gly-Gly-Lys-Gly-Gly-Asp-Leu-Tyr-Thr	陰	-
B-9	33-46	Met-Gly-Gly-Lys-Gly-Gly-Asp-Leu-Tyr-Thr-Val-Thr-Asn-Ser	性	-
B-10	37-50	Gly-Gly-Asp-Leu-Tyr-Thr-Val-Thr-Asn-Ser-Ser-Asp-Asp-Pro	陰	-
B-11	41-54	Tyr-Thr-Val-Thr-Asn-Ser-Ser-Asp-Asp-Pro-Val-Asn-Pro-Ala	性	-
B-12	45-58	Asn-Ser-Ser-Asp-Asp-Pro-Val-Asn-Pro-Ala-Pro-Gly-Thr-Leu	陰	-
B-13	49-62	Asp-Pro-Val-Asn-Pro-Ala-Pro-Gly-Thr-Leu-Arg-Tyr-Gly-Ala	性	-
B-14	53-66	Pro-Ala-Pro-Gly-Thr-Leu-Arg-Tyr-Gly-Ala-Thr-Arg-Asp-Arg	陰	-
B-15	57-70	Thr-Leu-Arg-Tyr-Gly-Ala-Thr-Arg-Asp-Arg-Pro-Leu-Trp-Ile	性	-
B-16	61-74	Gly-Ala-Thr-Arg-Asp-Arg-Pro-Leu-Trp-Ile-Ile-Phe-Ser-Gly	陰	-
B-17	65-78	Asp-Arg-Pro-Leu-Trp-Ile-Ile-Phe-Ser-Gly-Asn-Met-Asn-Ile	性	-
B-18	69-82	Trp-Ile-Ile-Phe-Ser-Gly-Asn-Met-Asn-Ile-Lys-Leu-Lys-Met	陰	-
B-19	73-86	Ser-Gly-Asn-Met-Asn-Ile-Lys-Leu-Lys-Met-Pro-Met-Tyr-Ile	性	-
B-20	77-90	Asn-Ile-Lys-Leu-Lys-Met-Pro-Met-Tyr-Ile-Ala-Gly-Tyr-Lys	陰	-
B-21	81-94	Lys-Met-Pro-Met-Tyr-Ile-Ala-Gly-Tyr-Lys-Thr-Phe-Asp-Gly	性	-
B-22	85-98	Tyr-Ile-Ala-Gly-Tyr-Lys-Thr-Phe-Asp-Gly-Arg-Gly-Ala-Gln	陰	-
B-23	89-102	Tyr-Lys-Thr-Phe-Asp-Gly-Arg-Gly-Ala-Gln-Val-Tyr-Ile-Gly	性	-
B-24	93-106	Asp-Gly-Arg-Gly-Ala-Gln-Val-Tyr-Ile-Gly-Asn-Gly-Gly-Pro	陰	-
B-25	97-110	Ala-Gln-Val-Tyr-Ile-Gly-Asn-Gly-Gly-Pro-Cys-Val-Phe-Ile	性	-
B-26	101-114	Ile-Gly-Asn-Gly-Gly-Pro-Cys-Val-Phe-Ile-Lys-Arg-Val-Ser	陰	-
B-27	105-118	Gly-Pro-Cys-Val-Phe-Ile-Lys-Arg-Val-Ser-Asn-Val-Ile-Ile	性	-
B-28	109-122	Phe-Ile-Lys-Arg-Val-Ser-Asn-Val-Ile-Ile-His-Gly-Leu-Tyr	陰	-
B-29	113-126	Val-Ser-Asn-Val-Ile-Ile-His-Gly-Leu-Tyr-Leu-Tyr-Gly-Cys	性	-
B-30	117-130	Ile-Ile-His-Gly-Leu-Tyr-Leu-Tyr-Gly-Cys-Ser-Thr-Ser-Val	陰	-
B-31	121-134	Leu-Tyr-Leu-Tyr-Gly-Cys-Ser-Thr-Ser-Val-Leu-Gly-Asn-Val	性	-
B-32	125-138	Gly-Cys-Ser-Thr-Ser-Val-Leu-Gly-Asn-Val-Leu-Ile-Asn-Glu	陰	-
B-33	129-142	Ser-Val-Leu-Gly-Asn-Val-Leu-Ile-Asn-Glu-Ser-Phe-Gly-Val	性	-
B-34	133-146	Asn-Val-Leu-Ile-Asn-Glu-Ser-Phe-Gly-Val-Glu-Pro-Val-His	陰	-
B-35	137-150	Asn-Glu-Ser-Phe-Gly-Val-Glu-Pro-Val-His-Pro-Gln-Asp-Gly	性	-

【表5】



B-36	141-154	Gly-Val-Glu-Pro-Val-His-Pro-Gln-Asp-Gly-Asp-Ala-Leu-Thr	性	-
B-37	145-158	Val-His-Pro-Gln-Asp-Gly-Asp-Ala-Leu-Thr-Leu-Arg-Thr-Ala	性	-
B-38	149-162	Asp-Gly-Asp-Ala-Leu-Thr-Leu-Arg-Thr-Ala-Thr-Asn-Ile-Trp	性	-
B-39	153-166	Leu-Thr-Leu-Arg-Thr-Ala-Thr-Asn-Ile-Trp-Ile-Asn-His-Asn	性	0.074 (0.045)
B-40	157-170	Thr-Ala-Thr-Asn-Ile-Trp-Ile-Asp-His-Asn-Ser-Phe-Ser-Asn	性	-
B-41	161-174	Ile-Trp-Ile-Asp-His-Asn-Ser-Phe-Ser-Asn-Ser-Ser-Asp-Gly	性	-
B-42	165-178	His-Asn-Ser-Phe-Ser-Asn-Ser-Ser-Asp-Gly-Leu-Val-Asp-Val	性	-
B-43	169-182	Ser-Asn-Ser-Ser-Asp-Gly-Leu-Val-Asp-Val-Thr-Leu-Thr-Ser	性	-
B-44	173-186	Asp-Gly-Leu-Val-Asp-Val-Thr-Leu-Thr-Ser-Thr-Gly-Val-Thr	性	-
B-45	177-190	Asp-Val-Thr-Leu-Thr-Ser-Thr-Gly-Val-Thr-Ile-Ser-Asn-Asn	性	-
B-46	181-194	Thr-Ser-Thr-Gly-Val-Thr-Ile-Ser-Asn-Asn-Leu-Phe-Asn	性	-
B-47	185-198	Val-Thr-Ile-Ser-Asn-Asn-Leu-Phe-Asn-His-His-Lys-Val	性	-
B-48	189-202	Asn-Asn-Leu-Phe-Asn-His-His-Lys-Val-Met-Leu-Leu-Gly	性	-
B-49	193-206	Phe-Asn-His-Lys-Val-Met-Leu-Leu-Gly-His-Asp-Asp-Ala	性	-
B-50	197-210	Lys-Val-Met-Leu-Leu-Gly-His-Asp-Asp-Ala-Tyr-Ser-Asp	性	-
B-51	201-214	Leu-Gly-His-Asp-Asp-Ala-Tyr-Ser-Asp-Asp-Lys-Ser-Met-Lys	性	-
B-52	205-218	Asp-Ala-Tyr-Ser-Asp-Asp-Lys-Ser-Met-Lys-Val-Thr-Val-Ala	性	-
B-53	209-222	Asp-Asp-Lys-Ser-Met-Lys-Val-Thr-Val-Ala-Phe-Asn-Gln-Phe	性	-
B-54	213-226	Met-Lys-Val-Thr-Val-Ala-Phe-Asn-Gln-Phe-Gly-Pro-Asn-Cys	性	-
B-55	217-230	Val-Ala-Phe-Asn-Gln-Phe-Gly-Pro-Asn-Cys-Gly-Gln-Arg-Met	性	-
B-56	221-234	Gln-Phe-Gly-Pro-Asn-Cys-Gly-Gln-Arg-Met-Pro-Arg-Ala-Arg	性	-
B-57	225-238	Asn-Cys-Gly-Gln-Arg-Met-Pro-Arg-Ala-Arg-Tyr-Gly-Leu-Val	性	-
B-58	229-242	Arg-Met-Pro-Arg-Ala-Arg-Tyr-Gly-Leu-Val-His-Val-Ala-Asn	性	-
B-59	233-246	Ala-Arg-Tyr-Gly-Leu-Val-His-Val-Ala-Asn-Asn-Tyr-Asp	性	-
B-60	237-250	Leu-Val-His-Val-Ala-Asn-Asn-Tyr-Asp-Pro-Trp-Thr-Ile	性	-
B-61	241-254	Ala-Asn-Asn-Tyr-Asp-Pro-Trp-Thr-Ile-Tyr-Ala-Ile-Gly	性	-
B-62	245-258	Tyr-Asp-Pro-Trp-Thr-Ile-Tyr-Ala-Ile-Gly-Gly-Ser-Ser-Asn	性	-
B-63	249-262	Thr-Ile-Tyr-Ala-Ile-Gly-Gly-Ser-Ser-Asn-Pro-Thr-Ile-Leu	性	-
B-64	253-266	Ile-Gly-Gly-Ser-Ser-Asn-Pro-Thr-Ile-Leu-Ser-Glu-Gly-Asn	性	-
B-65	257-270	Ser-Asn-Pro-Thr-Ile-Leu-Ser-Glu-Gly-Asn-Ser-Phe-Thr-Ala	性	-
B-66	261-274	Ile-Leu-Ser-Glu-Gly-Asn-Ser-Phe-Thr-Ala-Pro-Asn-Glu-Ser	性	-
B-67	265-278	Gly-Asn-Ser-Phe-Thr-Ala-Pro-Asn-Glu-Ser-Tyr-Lys-Lys-Gln	性	-
B-68	269-282	Thr-Ala-Pro-Asn-Glu-Ser-Tyr-Lys-Lys-Gln-Val-Thr-Ile-Arg	性	-

【表6】

B-69	273-286	Glu-Ser-Tyr-Lys-Lys-Gln-Val-Thr-Ile-Arg-Ile-Gly-Cys-Lys	性	-
B-70	277-290	Lys-Gln-Val-Thr-Ile-Arg-Ile-Gly-Cys-Lys-Thr-Ser-Ser-Ser	陰	-
B-71	281-294	Ile-Arg-Ile-Gly-Cys-Lys-Thr-Ser-Ser-Ser-Cys-Ser-Asn-Trp	陰	-
B-72	285-298	Cys-Lys-Thr-Ser-Ser-Ser-Cys-Ser-Asn-Trp-Val-Trp-Gln-Ser	陰	-
B-73	289-302	Ser-Ser-Cys-Ser-Ser-Asn-Trp-Val-Trp-Gln-Ser-Thr-Gln-Asp-Val	陰	-
B-74	293-306	Asn-Trp-Val-Trp-Gln-Ser-Thr-Gln-Asp-Val-Phe-Tyr-Asn-Gly	陰	-
B-75	297-310	Gln-Ser-Thr-Gln-Asp-Val-Phe-Tyr-Asn-Gly-Ala-Tyr-Phe-Val	陰	-
B-76	301-314	Asp-Val-Phe-Tyr-Asn-Gly-Ala-Tyr-Phe-Val-Ser-Ser-Gly-Lys	陰	-
B-77	305-318	Asn-Gly-Ala-Tyr-Phe-Val-Ser-Ser-Gly-Lys-Tyr-Glu-Gly-Gly	陰	-
B-78	309-322	Phe-Val-Ser-Ser-Gly-Lys-Tyr-Glu-Gly-Gly-Asn-Ile-Tyr-Thr	陰	-
B-79	313-326	Gly-Lys-Tyr-Glu-Gly-Gly-Asn-Ile-Tyr-Thr-Lys-Lys-Glu-Ala	陰	-
B-80	317-330	Gly-Gly-Asn-Ile-Tyr-Thr-Lys-Lys-Glu-Ala-Phe-Asn-Val-Glu	陰	0.077 (0.144)
B-81	321-334	Tyr-Thr-Lys-Lys-Glu-Ala-Phe-Asn-Val-Glu-Asn-Gly-Asn-Ala	陰	-
B-82	325-338	Glu-Ala-Phe-Asn-Val-Glu-Asn-Gly-Asn-Ala-Thr-Pro-Gln-Leu	陰	-
B-83	329-342	Val-Glu-Asn-Gly-Asn-Ala-Thr-Pro-Gln-Leu-Thr-Lys-Asn-Ala	陰	-
B-84	333-346	Asn-Ala-Thr-Pro-Gln-Leu-Thr-Lys-Asn-Ala-Gly-Val-Leu-Thr	陰	-
B-85	337-350	Gln-Leu-Thr-Lys-Asn-Ala-Gly-Val-Leu-Thr-Cys-Ser-Leu-Ser	陰	-
B-86	340-353	Lys-Asn-Ala-Gly-Val-Leu-Thr-Cys-Ser-Leu-Ser-Lys-Arg-Cys	陰	-
C-1	74-90	Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser-Trp-Lys-Asn	陽	0.084 (0.053)
C-2	91-107	Asn-Arg-Ile-Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly	陽	0.089 (0.076)
C-3	192-208	Asp-Ile-Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn-Thr-Ile-Gly-Thr	陽	0.071 (0.055)
C-4	352-368	Asp-Ile-Ser-Leu-Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn-Asp	陽	0.081 (0.083)
アミノ酸A	-	-	陽	1.281 (0.051)
アミノ酸B	-	-	陽	0.818 (0.075)

註：括弧内の数値は、陰性対照におけるM92を示す。

【0030】表1乃至6の結果は、試験に供したペプチド及びスギ花粉アレルゲンが、スギ花粉アレルゲンに特異的なT細胞に対して明らかに異なる挙動をしたことを示している。すなわち、試料A-19、A-20、A-23、A-48、A-49、A-89、B-39、B-

80又はスギ花粉アレルゲンA若しくはBを添加した系では、陰性対照と比較して、明らかに有意な<sup>3</sup>H-チミジンの取込み促進が認められたのに対して、その余の試料を添加した系においては、有意な取込み促進が認められなかった。このことは、試料A-19、A-20、A-

-23、A-48、A-49、A-89、B-39、B-80並びにスギ花粉アレルゲンA及びBのみが、単核細胞群中のスギ花粉アレルゲンに特異的なT細胞を有意に活性化したことを意味している。

【0031】さらに、アミノ酸配列が互いに重複する試料A-19、A-20、A-48及びA-49並びにT細胞活性化作用が他の陽性試料に比べてやや低かった試料A-23及びA-89につき、別途、17個のアミノ酸残基からなる、配列表における配列番号8乃至11に示すアミノ酸配列のペプチドを化学合成した。

【0032】すなわち、ミリジェン/バイオリサーチ製ペプチド合成機「エクセル」を使用し、常法にしたがって、配列表における配列番号8乃至11に示すアミノ酸配列のペプチド（試料C-1乃至C-4）を別々に合成し、バイオラッド製クロマトグラフィーカラム「Hi-Pore RP-318型」を使用する逆相高速液体クロマトグラフィーによりそれぞれ純度95%まで精製した。精製後、試料C-1乃至C-4の一部をとり、パーキン・エルマー製ペプチドシーケンサ「470A型」により分析したところ、合成に係る4種類のペプチドすべてが所期のアミノ酸配列を有していた。

【0033】これら試料C-1乃至C-4につき、上記と同様に試験したところ、いずれも陽性であり、スギ花粉アレルゲンに特異的なT細胞を有意に活性化することが判明した。

【0034】

【実験例2-2 スギ花粉アレルゲンに特異的なイムノグロブリンE抗体に対する反応性】実験例2-1においてスギ花粉アレルゲンに特異的なT細胞を有意に活性化することが明らかとなった試料B-39、B-80、C-1乃至C-4並びにアレルゲンA及びBに、タニアイらが「モレキュラー・イムノロジー」、第30巻、第2号、第183~189頁（1993年）に報告しているEIA法を適用し、スギ花粉症患者の血液から採取したスギ花粉アレルゲンに特異的なイムノグロブリンE抗体との反応性を調べた。

【0035】すなわち、ヒアス製架橋剤「（スルホスクシンイミジル）スベラート（BS<sub>3</sub>）」1gを蒸留水10mlに溶解し、メンク製「コバリンク型」96ウェルマイクロプレートに50μl/ウェルずつ分注し、37℃で3時間インキュベートした。蒸留水でマイクロプレートを洗浄し、実験例1及び2で調製した試料B-39、B-80、C-1乃至C-4又はアレルゲンA若しくはBを20μg/ml又は5μg/mlになるようにPBSに溶解し、マイクロプレートに50μl/ウェル分注し、37℃でさらに3時間インキュベートしてマイクロプレートに共有結合させた。そして、マイクロプレートに1%（w/v）ウシ血清アルブミンを含むPBSを50μl/ウェル加え、4℃で一晩静置して未反応の活性基をブロックした後、0.1%（w/v）ウシ血清

アルブミンを含むPBSで洗浄し、ウシ血清アルブミンを同量含む新鮮なPBSで5倍希釈したスギ花粉症患者の血清を50μl/ウェル加え、37℃で1時間反応させた。

【0036】次に、マイクロプレートを0.1%（w/v）ウシ血清アルブミンを含むPBSで洗浄し、ウシ血清アルブミンを同量含む新鮮なPBSで1μg/mlに希釈したキルケガード・アンド・ベリー製ビオチン標識抗ヒトε鎖抗体を50μl/ウェルずつ加え、37℃で1時間インキュベートした後、0.1%（w/v）ウシ血清アルブミンを含むPBSで再度洗浄後、ウシ血清アルブミンを同量含む新鮮なPBSで5,000倍に希釈したザイメッド製パーオキシダーゼ標識アビジンを50μl/ウェル加え、37℃でさらに1時間インキュベートした。そして、マイクロプレートを0.1%（w/v）ウシ血清アルブミンを含むPBSで洗浄後、過酸化水素を0.03%（v/v）とオルトフェニレンジアミンを0.5mg/ml含む0.1Mクエン酸-磷酸緩衝液（pH5.0）を100μl/ウェル加え、室温下で5分間静置して酵素反応させた。2N硫酸を100μl/ウェルずつ加えて反応を停止させた後、分光光度計を使用する公知の方法で492nmの波長下における吸光度を測定した。

【0037】同時に、スギ花粉患者の血清に代えて健康者の血清を使用する系を設け、同様に処置して陰性対照とした。結果を表1乃至6に示す。

【0038】表1乃至6に示す結果から明らかなように、アレルゲンA及びBがスギ花粉症患者由来のスギ花粉アレルゲンに特異的なイムノグロブリンE抗体に強く反応したのに対して、試料B-39、B-80及びC-1乃至C-4は実質的に反応しなかった。このことは、これら試料がアレルゲンA及びBに含まれるスギ花粉アレルゲンのB細胞エпитープを欠いていることを意味している。これらの結果と実験例2-1の結果を総合的に判断すると、上記試料、すなわち、配列表における配列番号8乃至13に示すアミノ酸配列のペプチドは、本質的にスギ花粉アレルゲンのT細胞エпитープからなるものであると判断される。

【0039】

【実験例3 T細胞がT細胞エпитープを認識するため不可欠なアミノ酸配列の検索】本実験例では、実験例2で明らかにした6種類のT細胞エпитープをさらに解析し、T細胞がそれらを認識するために不可欠なアミノ酸配列を検索した。

【0040】すなわち、実験例1-1の方法により、配列表における配列番号8乃至13に示すアミノ酸配列並びに表5乃至6において陽性を否定し切れなかった試料B-38、B-81及びB-82のアミノ酸配列につき、それらの一端又は両端のアミノ酸の1個又は2個以上をアラニンで置換したアミノ酸14個又は17個から

【0042】  
【表7】

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50 【0044】この発明のペプチドは、通常、5乃至50

個、望ましくは、10乃至20個のアミノ酸がペプチド結合してなる。個々のペプチドとしては、例えば、配列表における配列番号8乃至13に示すアミノ酸配列を有するものと、それらアミノ酸配列に相同的なアミノ酸配列を有するものが挙げられる。相同的なアミノ酸配列のペプチドは、上記の免疫学的作用を実質的に変えることなく、配列表における配列番号8乃至13に示すアミノ酸配列におけるアミノ酸の1個又は2個以上を他のアミノ酸で置換するか、それらアミノ酸配列の一端又は両端に適宜のアミノ酸を1個又は2個以上結合させることにより得ることができる。

【0045】具体的には、例えば、配列表の配列番号8乃至13に示すアミノ酸配列において、T細胞がそれらを認識するために不可欠なアミノ酸配列のみを不変とし、それ以外のアミノ酸については、スギ花粉のT細胞エпитープとしての免疫学的作用を実質的に変えない範囲で他のアミノ酸により置換する。あるいは、その不可欠なアミノ酸配列の一端又は両端に、必要に応じて、例えば、アラニンなどの適宜アミノ酸を1個又は2個以上結合させ、得られるペプチドが全体としてT細胞の認識し得る長さ、すなわち、通常、アミノ酸残基数にして10乃至20個になるようにする。斯かるアミノ酸配列としては、例えば、配列表における配列番号1乃至7に示すアミノ酸配列が挙げられ、また、斯かる相同体の例として、例えば、配列表における配列番号16乃至24に示すアミノ酸配列のペプチドを挙げることができる。

【0046】この発明のペプチドは、「固相法」又は「液相法」として知られる斯界において慣用のペプチド合成法により、容易に調製することができる。この発明はペプチド合成そのものに係わるものではないので、詳しい説明は省略するが、例えば、社団法人日本生化学会編「新生化学実験講座」、第1巻、「タンパク質V」I」、第3〜44頁、1992年、東京化学同人発行などにはペプチド合成の詳細が記載されている。ただし、この発明のペプチドは化学合成により調製されたものに限定されず、例えば、スギの花粉又は雄花から採取するか、組換えDNA技術により調製したスギ花粉アレルゲンを適宜分解し、分解物から採取したものであってもよい。あるいは、例えば、配列表における配列番号8乃至13に示すアミノ酸配列又はそれらに相同的なアミノ酸配列を有するペプチドをコードするDNAを調製し、これを自律複製可能なベクターに挿入して組換えDNAとし、これを大腸菌、枯草菌、放線菌、酵母などの適宜宿主に導入して形質転換体とし、その培養物からこの発明のペプチドを採取してもよい。配列表における配列番号8乃至13に示すアミノ酸配列のペプチドをコードするDNAは、例えば、特願平5-344596号明細書や国際特許公開第93/01213号明細書に記載されたcDNAの塩基配列に基づいて調製することができる。さらに、この発明のペプチドは、斯くして得られるペ

チドに糖質やポリエチレングリコールを付加して得られる複合体としての形態、さらには、ペプチドをアセチル化、アミド化及び／又は多官能試薬により架橋重合させて得られる誘導体又は重合体としての形態であってもよい。

【0047】この発明のペプチドは、比較的粗な形態で投与しても所期の治療・予防効果を発揮するが、通常は使用に先立って精製される。精製には、例えば、濾過、濃縮、遠心分離、ゲル濾過クロマトグラフィー、イオン交換クロマトグラフィー、高速液体クロマトグラフィー、アフィニティークロマトグラフィー、ゲル電気泳動、等電点電気泳動などのペプチド乃至蛋白質を精製するための斯界における慣用の方法が用いられ、必要に応じて、これら方法を適宜組合せればよい。そして、最終使用形態に応じて、精製したペプチドを濃縮・凍結乾燥して液状又は固状にすればよい。

【0048】前述のとおり、この発明のペプチドは、スギ花粉アレルゲンに特異的なイムノグロブリンE抗体に実質的に反応せず、しかも、スギ花粉アレルゲンに特異的なT細胞を有意に活性化するので、スギ花粉症を治療・予防するための免疫療法剤として広範な用途を有する。有効成分としてこの発明のペプチドを含んでなる免疫療法剤は、スギ花粉症に罹患したヒトを含む哺乳類一般に投与すると、アナフィラキシーなどの副作用を実質的に起こすことなく、スギ花粉症を治療することができる。一方、この発明の免疫療法剤を、スギ花粉が飛散し始める前に健康な個体や潜在的なスギ花粉症の個体に投与するときには、スギ花粉症に対して顕著な予防効果を発揮するとともに、発症時のアレルギー症状の緩解に著効を発揮する。

【0049】この発明の免疫療法剤につきさらに詳しく説明すると、この発明の免疫療法剤は、通常、この発明によるペプチドの1種又は2種以上を0.01乃至100% (w/w)、望ましくは、0.05乃至50% (w/w)、さらに望ましくは、0.5乃至5.0% (w/w) 含んでなる。この発明の免疫療法剤は、当該ペプチド単独の形態はもとより、それ以外の生理的に許容される、例えば、血清アルブミン、ゼラチン、マンニトール、マルトース、トレハロースなどの担体、賦形剤、免疫助成剤、安定剤、さらには、必要に応じて、ステロイドホルモンやクリモグリク酸ナトリウムなどの抗炎症剤や抗ヒスタミン剤を含む1種又は2種以上の他の薬剤との組成物としての形態を包含する。さらに、この発明の免疫療法剤は、投薬単位形態の薬剤をも包含し、その投薬単位形態の薬剤とは、この発明のポリペプチドを、例えば、1日当たりの用量又はその整数倍(4倍まで)又はその約数(1/40まで)に相当する量を含む、投与に適する物理的に分離した一体の剤型にある薬剤を意味する。このような投薬単位形態の薬剤としては、散剤、細粒剤、顆粒剤、丸剤、錠剤、カプセル剤、ロー

チ剤、シロップ剤、乳剤、軟膏剤、硬膏剤、パップ剤、坐剤、点眼剤、点鼻剤、噴霧剤、注射剤などが挙げられる。

【0050】この発明の免疫療法剤の使用法について説明すると、この発明の免疫療法剤は、スギ花粉症の治療・予防を目的に、ヒトを含む哺乳類一般に経皮、経口、点鼻、点眼又は注射投与される。ヒトにおける投与量は、投与の目的や症状に依っても変わるが、通常、対象者の症状や投与後の経過を観察しながら、成人1日当たり0.01乃至1.0g、望ましくは、0.01乃至0.1gを目安に、毎週1回乃至毎月1回の頻度で、約1乃至6カ月間、通常、用量を増やしながら反復投与される。

【0051】以下、この発明によるペプチドの調製と用途につき、2〜3の実施例を挙げて説明する。

【0052】

【実施例A-1 ペプチドの調製】ミリジェン/バイオリサーチ製ペプチド合成機「エクセル」を使用し、常法にしたがって、配列表における配列番号8乃至11に示すアミノ酸配列のペプチドを別々に合成し、バイオラド製クロマトグラフィーカラム「Hi-Pore RP-318型」を使用する逆相高速液体クロマトグラフィーによりそれぞれ純度95%まで精製後、凍結乾燥して固状物とした。固状物の一部をとり、パーキン・エルマー製ペプチドシーケンサ「470A型」により分析したところ、合成に係る4種類のペプチドすべてが所期のアミノ酸配列を有していた。

【0053】

【実施例A-2 ペプチドの調製】ケンブリッジ・リサーチ・バイオケミカルズ製ペプチド合成キット「マルチピン」を使用し、常法にしたがって、配列表における配列番号12及び13に示すアミノ酸配列のペプチドを別々に化学合成し、実施例A-1と同様にしてそれぞれ純度95%まで精製後、凍結乾燥して固状物とした。固状物の一部をとり、実施例A-1と同様に分析したところ、いずれも所期のアミノ酸配列を有していた。

【0054】

【実施例A-3 ペプチドの調製】実施例A-1と同様にして、配列表における配列番号16に示すアミノ酸配列のペプチドを化学合成し、純度95%まで精製した。精製後、ペプチドの一部をとり、実施例A-1と同様に分析したところ、所期のアミノ酸配列を有していた。

【0055】

【実施例A-4 ペプチドの調製】実施例A-2と同様にして、配列表における配列番号17に示すアミノ酸配列のペプチドを化学合成し、純度95%まで精製した。精製後、ペプチドの一部をとり、実施例A-1と同様に分析したところ、所期のアミノ酸配列を有していた。

【0056】

【実施例A-5 ペプチドの調製】実施例A-1乃至A

-2と同様にして、実験例3の試料D-1乃至D-7に相当する配列表における配列番号18乃至24に示すアミノ酸配列のペプチドを化学合成し、それぞれ純度95%まで精製後、凍結乾燥して固状物とした。固状物の一部をとり、パーキン・エルマー製ペプチドシーケンサ「470型」により分析したところ、合成に係る7種類のペプチドすべてが所期のアミノ酸配列を有していた。

【0057】

【実施例B-1 液剤】実施例A-1及びA-2の方法により得た6種類のペプチドのいずれかを最終濃度0.1g/mlになるように安定剤として1% (w/v) 精製ゼラチンを含む蒸留水に溶解し、常法により滅菌濾過して6種類の液剤を得た。

【0058】この発明のペプチドに対する感受性は、個体ごとに変わるのが通例であるから、本品は、個々の個体に最も適した組成になるよう、6種類の液剤を適宜配合して使用する。安定性に優れた本品は、スギ花粉症を治療・予防するための点眼剤、点鼻剤、口腔内噴霧剤用の液剤として有用である。

【0059】

【実施例B-2 注射剤】安定剤として1% (w/v) ヒト血清アルブミンを含む生理食塩水に実施例A-1及びA-2の方法により得た6種類のペプチドをそれぞれ最終濃度0.01、0.1又は1mg/mlになるように溶解し、滅菌濾過した後、滅菌バイアル瓶に2mlずつ分注し、凍結乾燥し、密栓した。

【0060】本品は、投与に先立ち、まず、バイアル瓶内に注射用蒸留水等を1ml加え、次いで、内容物を均一に溶解して使用する。安定性に優れ、有効成分としてこの発明による6種類のポリペプチドを含んでなる本品は、スギ花粉症を治療・予防するための乾燥注射剤として有用である。

【0061】

【実施例B-3 錠剤】平均分子量約20,000ダルトンの精製ブルラン2gを蒸留水100mlに均一に溶解し、溶液に塩化シアヌルの1.7% (w/v) アセトン溶液を2ml加え、5% (w/v) 炭酸ナトリウム水溶液でpHを7付近に保ちつつ、攪拌下、5℃で2時間反応させた。その後、同様にして反応物のpHを7付近に保ちながら、4℃の冷水に対して一晩透析し、活性化ブルランを含む水溶液20mlを得た。

【0062】この水溶液に実施例A-1の方法により得た配列表における配列番号8、10及び11に示すアミノ酸配列のペプチドと、実施例A-2の方法により得た配列表に配列番号13に示すアミノ酸配列のペプチドと、実施例A-3の方法により得たペプチドと、実施例A-4の方法により得たペプチドをそれぞれ0.2mg加え、溶液のpHを7付近に保ちつつ、緩やかに攪拌しながら、37℃で12時間反応させた。反応後、反応物にグリシンを4g加え、緩やかに攪拌しながら、37℃

で5時間インキュベートし、未反応の活性基をブロックした。

【0063】反応物を濃縮し、予め0.1M磷酸緩衝液(pH7.0)で平衡化させておいたセファデックスG-50カラムに負荷し、カラムに新鮮な同一緩衝液を通液して、この発明のペプチドとブルランの複合体を含む画分を採取した。収量は、原料ペプチド固形分当たり、約30%であった。

【0064】常法にしたがって、この画分を滅菌濾過し、濃縮し、凍結乾燥し、粉碎後、マンニトールを均一に混合し、混合物を打錠して製品1錠(200mg)当たり複合体を2.10又は50mg含む錠剤を得た。

【0065】摂取性、安定性に優れた本品は、スギ花粉症を治療・予防するための舌下剤として有用である。

【0066】

【実施例B-4 シロップ剤】大腸菌由来の精製リボ多糖1gを10mM磷酸カルシウム水溶液100mlに溶解し、溶液に100mM過沃素酸ナトリウムを6ml加え、室温下で20分間反応させてリボ多糖を活性化した。反応物を4℃の1Mグリシン-塩酸緩衝液(pH4.4)に対して一晩透析して未反応の過沃素酸を除去した後、0.1M炭酸水素ナトリウム緩衝液によりpH9.5付近に調整する一方、別途、実施例A-1及びA-2の方法により得た6種類のペプチドを0.1M磷酸緩衝液(pH7.0)100mlにそれぞれ10mgずつ溶解し、活性化リボ多糖を含む上記反応物に加え、室温下で12時間静置して反応させた。

【0067】その後、新たに得られた反応物を実施例B-3の方法により精製し、得られたこの発明のペプチドとリボ多糖の複合体を含む画分を濃縮し、凍結乾燥し、粉碎して固状物とした。収量は、原料ペプチド固形分当たり、約30%であった。

【0068】この固状物と蔗糖をそれぞれ最終濃度が0.1若しくは1mg/ml又は50%(w/w)になるように安定剤として精製ゼラチンを1%(w/v)含む蒸留水に溶解し、溶液を常法により滅菌濾過してシロップ状物を得た。このシロップ状物を2mlずつ滅菌バイアル瓶に分注し、密栓して製品とした。

【0069】安定性に優れ、有効成分としてこの発明のペプチドとリボ多糖の複合体を含む本品は、スギ花粉症を治療・予防するためのシロップ剤として有用である。

【0070】

【実施例B-5 液剤】実施例A-5の方法により得た7種類のペプチドのいずれかを最終濃度0.1g/mlになるように安定剤として1%(w/v)精製ゼラチンを含む蒸留水に溶解し、常法により滅菌濾過して7種類の液剤を得た。

【0071】この発明のペプチドに対する感受性は、個体ごとに変わるのが通例であるから、本品は、個々の個体に最も適した組成になるよう、7種類の液剤を適宜配

合して使用する。安定性に優れた本品は、スギ花粉症を治療・予防するための点眼剤、点鼻剤、口腔内噴霧剤用の液剤として有用である。

【0072】

【実施例B-6 注射剤】安定剤として1%(w/v)ヒト血清アルブミンを含む生理食塩水に実施例A-5の方法により得た7種類のペプチドをそれぞれ最終濃度0.01、0.1又は1mg/mlになるように溶解し、滅菌濾過した後、滅菌バイアル瓶に2mlずつ分注し、凍結乾燥し、密栓した。

【0073】本品は、投与に先立ち、先ず、バイアル瓶内に注射用蒸留水等を1ml加え、次いで、内容物を均一に溶解して使用する。安定性に優れ、有効成分としてこの発明による7種類のポリペプチドを含んでなる本品は、スギ花粉症を治療・予防するための乾燥注射剤として有用である。

【0074】

【実施例B-7 シロップ剤】精製ゼラチンを1%(w/v)含む蒸留水に実施例A-5の方法により得た7種類のペプチドをそれぞれ0.1mg/mlと蔗糖を50%(w/v)になるように溶解し、溶液を常法により滅菌濾過してシロップ状物を得た。このシロップ状物を2mlずつ滅菌バイアル瓶に分注し、密栓して製品とした。

【0075】安定性に優れ、有効成分としてこの発明のペプチドを含む本品は、スギ花粉症を治療・予防するためのシロップ剤として有用である。

【0076】

【実験例4 急性毒性試験】常法により、生後20日目のマウスに実施例B-1乃至B-7の方法により得た免疫療法剤を経口又は腹腔内投与した。その結果、これら免疫療法剤は、いずれの投与経路によっても200mg/kg以上のLD50であることが判明した。このことは、この発明のペプチドが、ヒトを含む哺乳類に投与する免疫療法剤に安全に配合使用し得ることを示している。

【0077】

【発明の効果】以上説明したように、この発明は、本質的にスギ花粉アレルゲンのT細胞エпитープからなるペプチドの発見に基づくものである。この発明のペプチドは、スギ花粉アレルゲンに特異的なイムノグロブリンE抗体に実質的に反応しないので、ヒトを含む哺乳類に投与すると、実質的にアナフィラキシーを引起することなく、スギ花粉アレルゲンに特異的なT細胞を活性化する。したがって、有効成分として斯かるペプチドを含んでなるこの発明の免疫療法剤は、ヒトを含む哺乳類に投与すると、副作用少なく、短期間でスギ花粉症に対して顕著な治療・予防効果を発揮する。しかも、この発明のペプチドは、所望量を容易に製造でき、品質管理も容易なことから、スギ花粉症の治療・予防にきわめて安全に

使用できるものである。

【0078】斯くも顕著な作用効果を発揮するこの発明は、斯界に貢献すること誠に多大な、意義のある発明と云える。

【0079】

【配列表】

配列番号：1

配列の長さ：11

配列の型：アミノ酸

トポロジー：直鎖状

配列の種類：ペプチド

配列

Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn

1 5 10

【0080】配列番号：2

配列の長さ：10

配列の型：アミノ酸

トポロジー：直鎖状

配列の種類：ペプチド

配列

Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser

1 5 10

【0081】配列番号：3

配列の長さ：10

配列の型：アミノ酸

トポロジー：直鎖状

配列の種類：ペプチド

配列

Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu

1 5 10

【0082】配列番号：4

配列の長さ：10

配列の型：アミノ酸

トポロジー：直鎖状

配列

Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser Trp Lys Asn

1 5 10 15

【0087】配列番号：9

配列の長さ：17

配列の型：アミノ酸

配列

Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu Met Gly

1 5 10 15

【0088】配列番号：10

配列の長さ：17

配列の型：アミノ酸

配列

Asp Ile Phe Ala Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr

1 5 10 15

【0089】配列番号：11

\* 配列の種類：ペプチド

配列

Phe Ala Ser Lys Asn Phe His Leu Gln Lys

1 5 10

【0083】配列番号：5

配列の長さ：11

配列の型：アミノ酸

トポロジー：直鎖状

配列の種類：ペプチド

10 配列

Ser Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser

1 5 10

【0084】配列番号：6

配列の長さ：8

配列の型：アミノ酸

トポロジー：直鎖状

配列の種類：ペプチド

配列

Leu Thr Leu Arg Thr Ala Thr Asn

20 1 5

【0085】配列番号：7

配列の長さ：5

配列の型：アミノ酸

トポロジー：直鎖状

配列の種類：ペプチド

配列

Ala Phe Asn Val Glu

1 5

【0086】配列番号：8

30 配列の長さ：17

配列の型：アミノ酸

トポロジー：直鎖状

配列の種類：ペプチド

\*

※トポロジー：直鎖状

配列の種類：ペプチド

※40

★トポロジー：直鎖状

配列の種類：ペプチド

★

50 配列の長さ：17



31

32

配列の型：アミノ酸  
トポロジー：直鎖状

\* 配列の種類：ペプチド

\*

配列

Asp Ile Ser Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn Asp

1

5

10

15

【0090】配列番号：12

※トポロジー：直鎖状

配列の長さ：14

配列の種類：ペプチド

配列の型：アミノ酸

※

配列

Leu Thr Leu Arg Thr Ala Thr Asn Ile Trp Ile Asp His Asn

1

5

10

【0091】配列番号：13

★トポロジー：直鎖状

配列の長さ：14

配列の種類：ペプチド

配列の型：アミノ酸

★

配列

Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu

1

5

10

【0092】配列番号：14

☆配列の種類：蛋白質

配列の長さ：514

起源

配列の型：アミノ酸

20 生物名：Cryptomeria japonica

トポロジー：直鎖状

☆ 個体・単離生物名：スギ

配列

Met Ala Met Lys Phe Ile Ala Pro Met Ala Phe Val Ala Met Gln Leu Ile

1

5

10

15

Ile Met Ala Ala Ala Glu Asp Gln Ser Ala Gln Ile Met Leu Asp Ser Asp

20

25

30

Ile Glu Gln Tyr Leu Arg Ser Asn Arg Ser Leu Arg Lys Val Glu His Ser

35

40

45

50

Arg His Asp Ala Ile Asn Ile Phe Asn Val Glu Lys Tyr Gly Ala Val Gly

55

60

65

Asp Gly Lys His Asp Cys Thr Glu Ala Phe Ser Thr Ala Trp Gln Ala Ala

70

75

80

85

Cys Lys Lys Pro Ser Ala Met Leu Leu Val Pro Gly Asn Lys Lys Phe Val

90

95

100

Val Asn Asn Leu Phe Phe Asn Gly Pro Cys Gln Pro His Phe Thr Phe Lys

105

110

115

Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser Trp Lys Asn Asn

120

125

130

135

Arg Ile Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu Met Gly Lys

140

145

150

Gly Val Ile Asp Gly Gln Gly Lys Gln Trp Trp Ala Gly Gln Cys Lys Trp

155

160

165

170

Val Asn Gly Arg Glu Ile Cys Asn Asp Arg Asp Arg Pro Thr Ala Ile Lys

175

180

185

Phe Asp Phe Ser Thr Gly Leu Ile Ile Gln Gly Leu Lys Leu Met Asn Ser

190

195

200

Pro Glu Phe His Leu Val Phe Gly Asn Cys Glu Gly Val Lys Ile Ile Gly

205

210

215

220

Ile Ser Ile Thr Ala Pro Arg Asp Ser Pro Asn Thr Asp Gly Ile Asp Ile

225

230

235

33 34  
 Phe Ala Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr Gly Asp  
 240 245 250 255  
 Asp Cys Val Ala Ile Gly Thr Gly Ser Ser Asn Ile Val Ile Glu Asp Leu  
 260 265 270  
 Ile Cys Gly Pro Gly His Gly Ile Ser Ile Gly Ser Leu Gly Arg Glu Asn  
 275 280 285  
 Ser Arg Ala Glu Val Ser Tyr Val His Val Asn Gly Ala Lys Phe Ile Asp  
 290 295 300 305  
 Thr Gln Asn Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly Ser Gly Met Ala  
 310 315 320  
 Ser His Ile Ile Tyr Glu Asn Val Glu Met Ile Asn Ser Glu Asn Pro Ile  
 325 330 335 340  
 Leu Ile Asn Gln Phe Tyr Cys Thr Ser Ala Ser Ala Cys Gln Asn Gln Arg  
 345 350 355  
 Ser Ala Val Gln Ile Gln Asp Val Thr Tyr Lys Asn Ile Arg Gly Thr Ser  
 360 365 370  
 Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys Ser Asp Ser Met Pro Cys Lys  
 375 380 385 390  
 Asp Ile Lys Leu Ser Asp Ile Ser Leu Lys Leu Thr Ser Gly Lys Ile Ala  
 395 400 405  
 Ser Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe Ser Gly His Val Ile Pro  
 410 415 420 425  
 Ala Cys Lys Asn Leu Ser Pro Ser Ala Lys Arg Lys Glu Ser Lys Ser His  
 430 435 440  
 Lys His Pro Lys Thr Val Met Val Lys Asn Met Gly Ala Tyr Asp Lys Gly  
 445 450 455  
 Asn Arg Thr Arg Ile Leu Leu Gly Ser Arg Pro Pro Asn Cys Thr Asn Lys  
 460 465 470 475  
 Cys His Gly Cys Ser Pro Cys Lys Ala Lys Leu Val Ile Val His Arg Ile  
 480 485 490  
 Met Pro Gln Glu Tyr Tyr Pro Gln Arg Trp Met Cys Ser Arg His Ala Lys  
 495 500 505 510  
 Ile Tyr His Pro

【 0 0 9 3 】 配列番号 : 15

配列の長さ : 353

配列の型 : アミノ酸

トポロジー : 直鎖状

\* 配列の種類 : 蛋白質

起源

生物名 : *Cryptomeria japonica*

\* 個体・単離生物名 : スギ

配列

Asp Asn Pro Ile Asp Ser Cys Trp Arg Gly Asp Ser Asn Trp Ala Gln Asn  
 1 5 10 15  
 Arg Met Lys Leu Ala Asp Cys Ala Val Gly Phe Gly Ser Ser Thr Met Gly  
 20 25 30  
 Gly Lys Gly Gly Asp Leu Tyr Thr Val Thr Asn Ser Asp Asp Asp Pro Val  
 35 40 45 50  
 Asn Pro Ala Pro Gly Thr Leu Arg Tyr Gly Ala Thr Arg Asp Arg Pro Leu  
 55 60 65  
 Trp Ile Ile Phe Ser Gly Asn Met Asn Ile Lys Leu Lys Met Pro Met Tyr  
 70 75 80 85  
 Ile Ala Gly Tyr Lys Thr Phe Asp Gly Arg Gly Ala Gln Val Tyr Ile Gly  
 90 95 100

35 36  
 Asn Gly Gly Pro Cys Val Phe Ile Lys Arg Val Ser Asn Val Ile Ile His  
 105 110 115  
 Gly Leu Tyr Leu Tyr Gly Cys Ser Thr Ser Val Leu Gly Asn Val Leu Ile  
 120 125 130 135  
 Asn Glu Ser Phe Gly Val Glu Pro Val His Pro Gln Asp Gly Asp Ala Leu  
 140 145 150  
 Thr Leu Arg Thr Ala Thr Asn Ile Trp Ile Asp His Asn Ser Phe Ser Asn  
 155 160 165 170  
 Ser Ser Asp Gly Leu Val Asp Val Thr Leu Thr Ser Thr Gly Val Thr Ile  
 175 180 185  
 Ser Asn Asn Leu Phe Phe Asn His His Lys Val Met Leu Leu Gly His Asp  
 190 195 200  
 Asp Ala Tyr Ser Asp Asp Lys Ser Met Lys Val Thr Val Ala Phe Asn Gln  
 205 210 215 220  
 Phe Gly Pro Asn Cys Gly Gln Arg Met Pro Arg Ala Arg Tyr Gly Leu Val  
 225 230 235  
 His Val Ala Asn Asn Asn Tyr Asp Pro Trp Thr Ile Tyr Ala Ile Gly Gly  
 240 245 250 255  
 Ser Ser Asn Pro Thr Ile Leu Ser Glu Gly Asn Ser Phe Thr Ala Pro Asn  
 260 265 270  
 Glu Ser Tyr Lys Lys Gln Val Thr Ile Arg Ile Gly Cys Lys Thr Ser Ser  
 275 280 285  
 Ser Cys Ser Asn Trp Val Trp Gln Ser Thr Gln Asp Val Phe Tyr Asn Gly  
 290 295 300 305  
 Ala Tyr Phe Val Ser Ser Gly Lys Tyr Glu Gly Gly Asn Ile Tyr Thr Lys  
 310 315 320  
 Lys Glu Ala Phe Asn Val Glu Asn Gly Asn Ala Thr Pro Gln Leu Thr Lys  
 325 330 335 340  
 Asn Ala Gly Val Leu Thr Cys Ser Leu Ser Lys Arg Cys  
 345 350

【0094】配列番号：16

配列の長さ：17

配列の型：アミノ酸

※トポロジー：直鎖状

配列の種類：ペプチド

\*

配列

Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu Gln Gly Phe Thr Leu Met Gly  
 1 5 10 15

【0095】配列番号：17

配列の長さ：14

配列の型：アミノ酸

※トポロジー：直鎖状

配列の種類：ペプチド

※

配列

Leu Ala Leu Arg Thr Ala Thr Asn Ile Trp Ile Asp His Asn  
 1 5 10

【0096】配列番号：18

配列の長さ：17

配列の型：アミノ酸

★トポロジー：直鎖状

配列の種類：ペプチド

★

配列

Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Ala Ala Ala Ala Ala Ala  
 1 5 10 15

【0097】配列番号：19

配列の長さ：17

配列の型：アミノ酸

50 トポロジー：直鎖状

配列の種類：ペプチド

配列

Ala Ala Ala Ala Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser Ala Ala Ala

1

5

10

15

【0098】配列番号：20

※トポロジー：直鎖状

配列の長さ：17

配列の種類：ペプチド

配列の型：アミノ酸

\*

配列

Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu Ala Ala Ala Ala Ala Ala

1

5

10

15

【0099】配列番号：21

※トポロジー：直鎖状

配列の長さ：17

配列の種類：ペプチド

配列の型：アミノ酸

※

配列

Ala Ala Phe Ala Ser Lys Asn Phe His Leu Gln Lys Ala Ala Ala Ala Ala

1

5

10

15

【0100】配列番号：22

★トポロジー：直鎖状

配列の長さ：17

配列の種類：ペプチド

配列の型：アミノ酸

★

配列

Ala Ala Ser Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser Ala Ala Ala Ala

1

5

10

15

【0101】配列番号：23

☆トポロジー：直鎖状

配列の長さ：14

配列の種類：ペプチド

配列の型：アミノ酸

☆

配列

Leu Thr Leu Arg Thr Ala Thr Asn Ala Ala Ala Ala Ala Ala

1

5

10

【0102】配列番号：24

◆トポロジー：直鎖状

配列の長さ：14

30 配列の種類：ペプチド

配列の型：アミノ酸

◆

配列

Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala Phe Asn Val Glu

1

5

10

フロントページの続き

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【手続補正書】  
 【提出日】平成13年9月5日(2001. 9. 5)  
 【手続補正1】  
 【補正対象書類名】明細書  
 【補正対象項目名】0016  
 【補正方法】変更  
 【補正内容】

【0016】本発明者らは、長年に亘るスギ花粉アレルギーに係わる研究の一成果として、昨年、スギ花粉アレルギーの主たる1成分が配列表における配列番号14に示すアミノ酸配列を有することを突止め、特願平5-344596号明細書(特開平7-170986号公報)に開示した。一方、国際特許公開第93/01213号明細書には、スギ花粉アレルギーの別の1成分が、配列表における配列番号15に示すアミノ酸配列を有すると開示されており、本発明者らも、平成6年4月14乃至16日に熊本県熊本市で開催された「第6回日本アレルギー学会春期臨床大会」において、同じアミノ酸配列を公表している。

【手続補正2】  
 【補正対象書類名】明細書  
 【補正対象項目名】0046  
 【補正方法】変更

【補正内容】

【0046】この発明のペプチドは、「固相法」又は「液相法」として知られる斯界において慣用のペプチド合成法により、容易に調製することができる。この発明はペプチド合成そのものに係わるものではないので、詳しい説明は省略するが、例えば、社団法人日本生化学会編「新生化学実験講座」、第1巻、「タンパク質V I」、第3〜44頁、1992年、東京化学同人発行などにはペプチド合成の詳細が記載されている。ただし、この発明のペプチドは化学合成により調製されたものに限定されず、例えば、スギの花粉又は雄花から採取するか、組換えDNA技術により調製したスギ花粉アレルギーを適宜分解し、分解物から採取したものであってもよい。あるいは、例えば、配列表における配列番号8乃至13に示すアミノ酸配列又はそれらに相同的なアミノ酸配列を有するペプチドをコードするDNAを調製し、これを自律複製可能なベクターに挿入して組換えDNAとし、これを大腸菌、枯草菌、放線菌、酵母などの適宜宿主に導入して形質転換体とし、その培養物からこの発明のペプチドを採取してもよい。配列表における配列番号8乃至13に示すアミノ酸配列のペプチドをコードするDNAは、例えば、特願平5-344596号明細書

(特開平 7-170986 号公報) や国際特許公開第 93/01213 号明細書に記載された cDNA の塩基配列に基づいて調製することができる。さらに、この発明のペプチドは、斯くして得られるペプチドに糖質やポリ

エチレングリコールを付加して得られる複合体としての形態、さらには、ペプチドをアセチル化、アミド化及び／又は多官能試薬により架橋重合させて得られる誘導体又は重合体としての形態であってよい。

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(54) PEPTIDE AND ITS USE

(57)Abstract:

PURPOSE: To obtain a peptide not reacting with IgE specific to cedar pollen allergen, capable of activating a

THE PRESENT INVENTION RELATES TO A PEPTIDE AND ITS USE. THE INVENTION IS BASED ON THE FINDING THAT A PEPTIDE HAVING A SPECIFIC SEQUENCE DOES NOT REACT WITH AN ANTIBODY SPECIFIC TO CEDAR POLLEN ALLERGEN, BUT CAN ACTIVATE A CELL. THE INVENTION PROVIDES A PEPTIDE HAVING A SPECIFIC SEQUENCE, AND A METHOD FOR PRODUCING THE SAME. THE INVENTION ALSO PROVIDES A METHOD FOR USING THE SAME.

T cell specific to cedar pollen allergen by judging taking-in of 3H-thymidine and useful as an immunotherapeutic agent for treating cedar pollinosis, etc. CONSTITUTION: This new peptide contains either one of amino acid sequences expressed by formulas I to VII and does not react with immunoglobulin E(IgE) antibody specific to cedar pollen allergen and significantly activates a T cell specific to cedar pollen allergen, compared with negative reference examination using a method to judge taking in of

Lys Val Asp Gly His His Ala Ala Tyr Cys Arg	I
Tyr His Ala Ala Gly Gly Arg His His Ser	II
Arg Arg His Thr Leu His His Ala Lys Leu	III
Phe Ala Ser Leu Asp Phe His Leu Gly Lys	IV
Ser Leu Lys Leu Thr Ser Gly Lys His Ala Ser	V
Leu Thr Leu Arg Phe His Thr Arg	VI
Ala Phe Asn Val Gly	VII

3H-thymidine and exhibits remarkable treating and preventing effect on cedar pollen in a short period without almost giving adverse effect due to administering to Mammalia including Human as an active ingredient for immunotherapeutic agents. The peptide is obtained by extracting pollen collected from male flower of cedar by immersing it in 0.125M aqueous solution of sodium hydrogen carbonate and purifying the extracted solution by salting out using ammonium sulfate, treatments with anion exchanger and by gel filtration, etc., or by recombinant gene technique.

## LEGAL STATUS

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- 1.This document has been translated by computer. So the translation may not reflect the original precisely.
- 2.\*\*\*\* shows the word which can not be translated.
- 3.In the drawings, any words are not translated.

**DETAILED DESCRIPTION**

[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to the immunotherapy agent which comes to contain a new peptide, the peptide which activates a specific T cell to that application, division, and cedar pollen allergen, and its peptide as an active principle.

[0002]

[Description of the Prior Art] If it has become at the beginning of spring in our country since here about ten years, the number of those who appeal against the rhinitis and the conjunctivitis by hay fever will continue increasing. Since it is called the beginning of spring when there being many patients and an event with various \*\*\*\*\* continue, mass communications are also taken up frequently and, now, it has become one of the problems which cannot be disregarded on public health.

[0003] Hay fever is a kind of an allergy and it is said that the main factor is, the antigenic matter, i.e., the cedar pollen allergen, in cedar pollen. If the cedar pollen which dispersed in atmospheric air trespasses upon the human inside of the body, the immunoglobulin E antibody to cedar pollen allergen will produce. When cedar pollen invades next in this condition, the allergen and this immunoglobulin E antibody in that pollen will cause an immunoreaction, and will present an allergy symptom.

[0004] It is known that at least two kinds of allergen from which antigenic is different exists in current and cedar pollen. One of them is allergen which YASUEDA and others has reported to "journal OBU allergy - and - clinical immunology", the 71st volume, No. 1, and the 77-86th page (1983), and this is called "Cryj I" today. Another is TANIAI et al. "EFU I BI S Letters", the 239th volume, No. 2, the 329-332nd page (1988) and Sakaguchi "allergy" et al., No. 45, and allergen reported to the 309-312nd page (1990), and this is called "Cry j II" today. In cedar pollen, it is usually Cry. j I and Cry j Most blood serums

which II existed at a rate of about 50:1 thru/or 5:1, and extracted from the hay fever sufferer are Cry. It is Cry also to jI. j It is said that it reacts also to II. Sawatani and others -- "allergy", the 42nd volume, No. 6, and the 738-747th page (1993) -- setting -- Cryj II -- intracutaneous testing -- if a RAST trial is carried out -- Cry j It is reported that it demonstrates antigenic [ comparable as I ].

[0005] Thus, since cedar pollen allergen was already isolated partly and the property and description were also solved to some extent, the prospect which can treat and prevent hay fever followed by prescribing for the patient and carrying out hyposensitization of the purification cedar pollen allergen to Homo sapiens. Recently, it succeeds in the proposal with which carries out covalent bond of the sugar to the cedar pollen allergen as which some hyposensitization agents for it are also devised, for example, the amino acid sequence from an amino terminal is expressed in Asp-Asn-Pro-Ile-Asp-Ser or Ala-Ile-Asn-Ile-Phe-Asn to JP,1-156926,A or JP,3-93730,A, and Homo sapiens is medicated by making the generated complex into a hyposensitization agent. However, it is expected that great difficulty will follow on a diagnosis and desensitization therapy of an allergy if the allergen in cedar pollen has low stability to a small top and tends to provide the diagnostic agent and hyposensitization agent of hay fever only with cedar pollen to it the place for which the allergen of a high grade is usually needed in large quantities.

[0006] Since it is such, in the therapy and prevention of the latest allergosis, like the former, a patient is not medicated with the whole allergen but the minimum area which the T cell in allergen recognizes specifically, i.e., the immunotherapy which prescribes for the patient the low-molecular peptide which essentially consists of a T cell epitope, is capturing the spotlight.

[0007] Generally, when allergen is incorporated by antigen presenting cells, such as a macrophage, it is digested there, and a digestive fragment will join together and antigen presentation will be carried out to the HLA protein of an immunity presentation cell cortex. The field which the fragment by which antigen presentation is carried out is restricted to some [ in allergen ] specific regions with the compatibility over HLA protein etc., and a T cell recognizes specifically among these fields is usually called a "T cell epitope." In the immunotherapy which prescribes for the patient the peptide which essentially consists of a T cell epitope (i) The peptide lacks the B cell epitope, namely, since a specific immunoglobulin E antibody does not react to allergen, side effects, such as anaphylaxis which had occurred frequently with the conventional poor quality or purification allergen, cannot happen.

(ii) It starts from small quantity and a period until it reaches an effective dose can be sharply shortened as compared with the conventional hyposensitization agent. There is which advantage.

[0008] At present, although the T cell epitope of various allergen is analyzed

energetically, as far as all the amino acid sequences of allergen usually become indispensable at the analysis of a T cell epitope and cedar pollen allergen is concerned at least, the actual condition is that a T cell epitope has come to be solved substantially.

[0009]

[Problem(s) to be Solved by the Invention] The first technical problem of this invention is in view of this situation to offer the peptide which essentially consists of a T cell epitope of cedar pollen allergen, and a peptide homonous to it.

[0010] The second technical problem of this invention is to offer the immunotherapy agent which comes to contain the above-mentioned peptide as an active principle.

[0011]

[Means for Solving the Problem] This invention will solve a specific T cell with the peptide activated intentionally to cedar pollen allergen as compared with a negative control, if said first technical problem is examined by the approach which does not react to an immunoglobulin E antibody specific to cedar pollen allergen substantially, but is judged by the incorporation of 3H-thymidine.

[0012] This invention solves said second technical problem by the immunotherapy agent which comes to contain this peptide as an active principle.

[0013]

[Embodiment of the Invention] The peptide of this invention activates a specific T cell to cedar pollen allergen, without causing anaphylaxis substantially, if the general mammals including Homo sapiens are medicated since it does not react to an immunoglobulin E antibody specific to cedar pollen allergen substantially.

[0014] The immunotherapy agent of this invention that comes to contain this peptide as an active principle demonstrates remarkable therapy and preventive effect to hay fever, without causing anaphylaxis substantially, if the general mammals including Homo sapiens are medicated.

[0015] Hereafter, this invention is based on discovery of the peptide which essentially consists of a T cell epitope of cedar pollen allergen that the example of an experiment, an example, etc. explain this invention.

[0016] As one result of the research concerning the cedar pollen allergen over many years, this invention persons traced having the amino acid sequence which one main component of cedar pollen allergen shows to the array number 14 in an array table, and indicated on the Japanese-Patent-Application-No. No. 344596 [ five to ] specifications last year. On the other hand, if one another component of cedar pollen allergen has the amino acid sequence shown in the array number 15 in an array table on international patent public presentation/[ 93rd ] No. 01213 specifications, it will be indicated by them, and

this invention persons have also announced to them the same amino acid sequence in the "6th Japanese Society of Allergology spring clinical convention" held in Kumamoto, Kumamoto on 14 thru/or the 16th in April, Heisei 6.

[0017] Then, this invention person compounded the peptide of the amino acid sequence which is mutually [ about 180 kind ] different which consists of continuous 11 in these amino acid sequences, 14, or 17 amino acid residue based on the amino acid sequence shown in these array numbers 14 and 15 that the T cell epitope of cedar pollen allergen should be solved, and examined per [ to the reactivity over an immunoglobulin E antibody specific to cedar pollen allergen, and a T cell specific to cedar pollen allergen ] activation operation. Consequently, when the peptide which has the amino acid sequence shown in the array number 8 in an array table thru/or 13 was examined by the approach which does not react to an immunoglobulin E antibody specific to cedar pollen allergen substantially, and is judged by the incorporation of 3H-thymidine, it became clear [ activating a specific T cell intentionally to cedar pollen allergen ] as compared with the negative control. This has suggested that it is what the peptide which has the amino acid sequence shown in these array number 8 thru/or 13 essentially becomes from the T cell epitope of cedar pollen allergen. Moreover, when the amino acid sequence shown in the array number 8 thru/or 13 was analyzed further, it became clear that the amino acid sequence shown in the array number 1 of an array table thru/or 7 was an array indispensable in order that a T cell may recognize the peptide of the amino acid sequence shown in the array number 8 thru/or 13.

[0018] The following examples 1 and 2 of an experiment explain [ which came to solve these facts ] a series of experiments.

[0019]

[Preparation of an example of experiment 1 peptide, and cedar pollen allergen]

[0020]

[Preparation of an example of experiment 1-1 peptide] It is known as above-mentioned that at least two kinds of allergen from which a property and description are different exists in cedar pollen until now. The maturation protein of these cedar pollen allergen by recombinant DNA technology It is shown clearly that it has the amino acid sequence shown in the array number 14 in an array table or 15. Actually from cedar pollen Cedar pollen allergen of the amino acid sequence equivalent to the 46th in the amino acid sequence shown in the array number 14 thru/or the 433rd or the 51st thru/or the 433rd (it is hereafter called "Allergen A".) Cedar pollen allergen which has the 1st thru/or the 353rd amino acid sequence in the amino acid sequence shown in the array number 15 (it is hereafter called "Allergen B".) It is isolated. In addition, in the gene which carries out the code of the allergen A, since transit peptide is not decided, in the array number 14, the sign "1" is still provisionally

given to the amino acid residue of the beginning by the side of the amino terminal in the amino acid sequence decoded from the base sequence of cDNA.

[0021] In this example of an experiment, about the amino acid sequence shown in the array number 14 in an array table Covering the 46th thru/or 433rd field, and overlapping amino acid residue ten pieces at a time While carrying out chemosynthesis of the peptide (a sample A-1 thru/or A-95) of 95 kinds of different amino acid sequences which consist of 11 or 14 amino acid residue About the amino acid sequence shown in the array number 15 Covering the 1st thru/or 353rd field, and overlapping amino acid residue ten pieces at a time similarly Chemosynthesis of the peptide (a sample B-1 thru/or B-86) of 86 kinds of different amino acid sequences which consist of 14 amino acid residue was carried out, and the example 2 of the after-mentioned experiment for searching the peptide of this invention was presented.

[0022] That is, it checked consisting of 11 or 14 amino acid residue, compounding 181 kinds of peptides which have the amino acid sequence shown in the after-mentioned table 1 thru/or 6 according to a conventional method, by the solid phase technique which uses the peptide synthesis kit made from the Cambridge research biochemicals "multipin", taking the part after composition, and the Perkin-Elmer peptide sequencer "470A molds" analyzing, and having the expected amino acid sequence.

[0023]

[Preparation of example of experiment 1-2 cedar pollen allergen] It extracted at 4 degrees C for 1 hour, having been immersed in the 0.125M sodium-hydrogencarbonate water solution (pH8.2) of the about 16 weight section, and agitating quietly the pollen 1 weight section extracted from the male of URASUGI from Akita Prefecture. It put [ so that it may put at 4 degrees C for 1 hour, agitating / so that the supernatant liquid of the supernatant liquid which carried out centrifugal separation of the extract, extracted residue again like the above, and was obtained, and the first time may be pooled and it may become 0.1% (w/v) to this about cetavlon / gently, polysaccharide may be settled and it may become 80% saturation about an ammonium sulfate after centrifugal separation and at supernatant liquid ] at 4 degrees C one whole day and night, and salted out.

[0024] The precipitation section in a salting-out object is extracted, this was dialyzed to the 50mM tris-hydrochloric-acid buffer solution (pH7.8) for 10 hours, and elution of the fraction which carries out a load to the DEAE-sephadex column made to equilibrate with the 50mM tris-hydrochloric-acid buffer solution (pH7.8) beforehand, dips the same buffer solution fresh to a column, and contains a protein component was carried out after filtration. This fraction was extracted, the load was carried out to CM-sephadex column which added the acetic acid and was made to equilibrate

with 10mM acetic-acid buffer solution (pH5.0) beforehand after adjusting to pH5.0, 0.1M phosphate buffer (pH7.0) which contains 0.3M sodium chloride in a column for a column after washing with 10mM acetic-acid buffer solution (pH5.0) was dipped, and the fraction containing a protein component was extracted.

[0025] Next, Mono which this fraction was made to equilibrate with 10mM acetic-acid buffer solution (pH5.0) beforehand A load is carried out to S column. Under the concentration gradient of the sodium chloride which goes up a column from 0M to 0.5M after washing with 10mM acetic-acid buffer solution (pH5.0), the place which dipped 10mM phosphate buffer (pH7.0) in the column -- the sodium chloride concentration of the 0.1 thru/or 0.3 neighborhoods -- Allergen B -- moreover, Allergen A was eluted by the sodium chloride concentration near 0.4M. The fraction containing Allergen A and B was extracted separately, suitably, after concentration, it freeze-dried and the following example 2 of an experiment was presented. Yield was about 0.02% with Allergen B about 0.01% per raw material cedar pollen solid content and in Allergen A.

[0026]

[Retrieval of the peptide containing the T cell epitope of example of experiment 2 cedar pollen allergen]

[0027]

[Activation of a T cell specific to example of experiment 2-1 cedar pollen allergen] By the Ficoll-Hypaque-gradient-centrifugation method, the mononuclear cell group which contains a specific T cell in cedar pollen allergen was separated from a hay fever sufferer's heparinized peripheral blood. RPMI1640 culture medium (pH7.0) which supplemented this mononuclear cell group with AB blood serum 5% (v/v) is made to float. It pours distributively  $5 \times 10^5$  pieces / well every on 96 well microplate. After making into 200microl / well the peptide prepared by the example 1-1 of an experiment, and 1-2, or cedar pollen allergen by 1microg / well \*\*\*\*, and the same fresh culture medium, it incubated for two days at 37 degrees C among the 5%CO<sub>2</sub> incubator. Then, after adding 3H-thymidine 1.0microcurie / well every and incubating under the same conditions for further 16 hours, the amount of incorporation of the 3H-thymidine in a mononuclear cell group was measured by the well-known approach of using a scintillation counter. The system which does not contain peptide or cedar pollen allergen in coincidence, either was prepared, a measure was taken like the above, and it considered as the negative control.

[0028] The existence of an activation operation to a T cell specific to cedar pollen allergen was judged based on the amount (cpm) of incorporation of the 3H-thymidine in the mononuclear cell group containing this T cell, made the "positivity" the system to which the amount of incorporation reached the

twice [ more than ] as many abbreviation for a negative control as this, and made "negative" the system which was not attained. A result is shown in Table 1 thru/or 6.

[0029]

[Table 1]



試料	位置	アミノ酸配列	T細胞の活性化	ヒトイムノグロブリンE抗体 に対する反応性 (A492)
A-1	46-59	Arg-Lys-Val-Glu-His-Ser-Arg-His-Asp-Ala-Ile-Asn-Ile-Phe	陰性	-
A-2	51-64	Ser-Arg-His-Asp-Ala-Ile-Asn-Ile-Phe-Asn-Val-Glu-Lys-Tyr	陰性	-
A-3	55-68	Ala-Ile-Asn-Ile-Phe-Asn-Val-Glu-Lys-Tyr-Gly-Ala-Val-Gly	陰性	-
A-4	59-72	Phe-Asn-Val-Glu-Lys-Tyr-Gly-Ala-Val-Gly-Asp-Gly-Lys-His	陰性	-
A-5	63-76	Lys-Tyr-Gly-Ala-Val-Gly-Asp-Gly-Lys-His-Asp-Cys-Thr-Glu	陰性	-
A-6	67-80	Val-Gly-Asp-Gly-Lys-His-Asp-Cys-Thr-Glu-Ala-Phe-Ser-Thr	陰性	-
A-7	71-84	Lys-His-Asp-Cys-Thr-Glu-Ala-Phe-Ser-Thr-Ala-Trp-Gln-Ala	陰性	-
A-8	75-88	Thr-Glu-Ala-Phe-Ser-Thr-Ala-Trp-Gln-Ala-Ala-Cys-Lys-Lys	陰性	-
A-9	79-92	Ser-Thr-Ala-Trp-Gln-Ala-Ala-Cys-Lys-Lys-Pro-Ser-Ala-Met	陰性	-
A-10	83-96	Gln-Ala-Ala-Cys-Lys-Lys-Pro-Ser-Ala-Met-Leu-Leu-Val-Pro	陰性	-
A-11	87-100	Lys-Lys-Pro-Ser-Ala-Met-Leu-Leu-Val-Pro-Gly-Asn-Lys-Lys	陰性	-
A-12	91-104	Ala-Met-Leu-Leu-Val-Pro-Gly-Asn-Lys-Lys-Phe-Val-Val-Asn	陰性	-
A-13	95-108	Val-Pro-Gly-Asn-Lys-Lys-Phe-Val-Val-Asn-Asn-Leu-Phe-Phe	陰性	-
A-14	99-112	Lys-Lys-Phe-Val-Val-Asn-Asn-Leu-Phe-Phe-Asn-Gly-Pro-Cys	陰性	-
A-15	103-116	Val-Asn-Asn-Leu-Phe-Phe-Asn-Gly-Pro-Cys-Gln-Pro-His-Phe	陰性	-
A-16	107-120	Phe-Phe-Asn-Gly-Pro-Cys-Gln-Pro-His-Phe-Thr-Phe-Lys-Val	陰性	-
A-17	111-124	Pro-Cys-Gln-Pro-His-Phe-Thr-Phe-Lys-Val-Asp-Gly-Ile-Ile	陰性	-
A-18	115-128	His-Phe-Thr-Phe-Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln	陰性	-
A-19	119-132	Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser	陰性	-
A-20	123-136	Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser-Trp-Lys-Asn-Asn	陽性	-
A-21	127-140	Tyr-Gln-Asn-Pro-Ala-Ser-Trp-Lys-Asn-Asn-Arg-Ile-Trp-Leu	陽性	-
A-22	131-144	Ala-Ser-Trp-Lys-Asn-Asn-Arg-Ile-Trp-Leu-Gln-Phe-Ala-Lys	陰性	-
A-23	135-148	Asn-Asn-Arg-Ile-Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe	陰性	-
A-24	139-152	Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly	陽性	-
A-25	143-156	Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly-Lys-Gly-Val-Ile	陰性	-
A-26	147-160	Gly-Phe-Thr-Leu-Met-Gly-Lys-Gly-Val-Ile-Asp-Gly-Gln-Gly	陰性	-
A-27	151-164	Met-Gly-Lys-Gly-Val-Ile-Asp-Gly-Gln-Gly-Lys-Gln-Trp-Trp	陰性	-
A-28	155-168	Val-Ile-Asp-Gly-Gln-Gly-Lys-Gln-Trp-Trp-Ala-Gly-Gln-Cys	陰性	-
A-29	159-172	Gln-Gly-Lys-Gln-Trp-Trp-Ala-Gly-Gln-Cys-Lys-Trp-Val-Asn	陰性	-
A-30	163-176	Trp-Trp-Ala-Gly-Gln-Cys-Lys-Trp-Val-Asn-Gly-Arg-Glu-Ile	陰性	-
A-31	167-180	Gln-Cys-Lys-Trp-Val-Asn-Gly-Arg-Glu-Ile-Cys-Asn-Asp-Arg	陰性	-

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
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[Table 2]

A-32	171-184	Val-Asn-Gly-Arg-Glu-Ile-Cys-Asn-Asp-Arg-Asp-Arg-Pro-Thr	性	-
A-33	175-188	Glu-Ile-Cys-Asn-Asp-Arg-Arg-Pro-Thr-Ala-Ile-Lys-Phe	陰	-
A-34	179-192	Asp-Arg-Asp-Arg-Pro-Thr-Ala-Ile-Lys-Phe-Asp-Phe-Ser-Thr	性	-
A-35	183-196	Pro-Thr-Ala-Ile-Lys-Phe-Asp-Phe-Ser-Thr-Gly-Leu-Ile-Ile	陰	-
A-36	187-200	Lys-Phe-Asp-Phe-Ser-Thr-Gly-Leu-Ile-Ile-Gln-Gly-Leu-Lys	性	-
A-37	191-204	Ser-Thr-Gly-Leu-Ile-Ile-Gln-Gly-Leu-Lys-Leu-Met-Asn-Ser	陰	-
A-38	185-208	Ile-Ile-Gln-Gly-Leu-Lys-Leu-Met-Asn-Ser-Pro-Glu-Phe-His	陰	-
A-39	199-212	Leu-Lys-Leu-Met-Asn-Ser-Pro-Glu-Phe-His-Leu-Val-Phe-Gly	性	-
A-40	203-216	Asn-Ser-Pro-Glu-Phe-His-Leu-Val-Phe-Gly-Asn-Cys-Gln-Gly	陰	-
A-41	207-220	Phe-His-Leu-Val-Phe-Gly-Asn-Cys-Glu-Gly-Val-Lys-Ile-Ile	性	-
A-42	211-224	Phe-Gly-Asn-Cys-Glu-Gly-Val-Lys-Ile-Ile-Gly-Ile-Ser-Ile	陰	-
A-43	215-228	Glu-Gly-Val-Lys-Ile-Ile-Gly-Ile-Ser-Ile-Thr-Ala-Pro-Arg	陰	-
A-44	219-232	Ile-Ile-Gly-Ile-Ser-Ile-Thr-Ala-Pro-Arg-Asp-Ser-Pro-Asn	性	-
A-45	223-236	Ser-Ile-Thr-Ala-Pro-Arg-Asp-Ser-Pro-Asn-Thr-Asp-Gly-Ile	陰	-
A-46	227-240	Pro-Arg-Asp-Ser-Pro-Asn-Thr-Asp-Gly-Ile-Asp-Ile-Phe-Ala	陰	-
A-47	231-244	Pro-Asn-Thr-Asp-Gly-Ile-Asp-Ile-Phe-Ala-Ser-Lys-Asn-Phe	性	-
A-48	235-248	Gly-Ile-Asp-Ile-Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys	性	-
A-49	239-252	Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn-Thr-Ile-Gly	陽	-
A-50	243-256	Asn-Phe-His-Leu-Gln-Lys-Asn-Thr-Ile-Gly-Thr-Gly-Asp-Asp	陽	-
A-51	247-260	Gln-Lys-Asn-Thr-Ile-Gly-Thr-Gly-Asp-Asp-Cys-Val-Ala-Ile	陰	-
A-52	251-264	Ile-Gly-Thr-Gly-Asp-Asp-Cys-Val-Ala-Ile-Gly-Thr-Gly-Ser	陰	-
A-53	255-268	Asp-Asp-Cys-Val-Ala-Ile-Gly-Thr-Gly-Ser-Ser-Asn-Ile-Val	陰	-
A-54	269-272	Ala-Ile-Gly-Thr-Gly-Ser-Ser-Asn-Ile-Val-Ile-Glu-Asp-Leu	陰	-
A-55	263-276	Gly-Ser-Ser-Asn-Ile-Val-Ile-Glu-Asp-Leu-Ile-Cys-Gly-Pro	陰	-
A-56	267-280	Ile-Val-Ile-Glu-Asp-Leu-Ile-Cys Gly-Pro-Gly-His-Gly-Ile	陰	-
A-57	271-284	Asp-Leu-Ile-Cys-Gly-Pro-Gly-His-Gly-Ile-Ser-Ile-Gly-Ser	陰	-
A-58	275-288	Gly-Pro-Gly-His-Gly-Ile-Ser-Ile-Gly-Ser-Leu-Gly-Arg-Glu	陰	-
A-59	279-292	Gly-Ile-Ser-Ile-Gly-Ser-Leu-Gly-Arg-Glu-Asn-Ser-Arg-Ala	陰	-
A-60	283-296	Gly-Ser-Leu-Gly-Arg-Glu-Asn-Ser-Arg-Ala-Glu-Val-Ser-Tyr	陰	-
A-61	287-300	Arg-Glu-Asn-Ser-Arg-Ala-Glu-Val-Ser-Tyr-Val-His-Val-Asn	陰	-
A-62	291-304	Arg-Ala-Glu-Val-Ser-Tyr-Val-His-Val-Asn-Gly-Ala-Lys-Phe	陰	-
A-63	295-308	Ser-Tyr-Val-His-Val-Asn-Gly-Ala-Lys-Phe-Ile-Asp-Thr-Gln	陰	-
A-64	299-312	Val-Asn-Gly-Ala-Lys-Phe-Ile-Asp-Thr-Gln-Asn-Gly-Leu-Arg	陰	-

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
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[Table 3]

A-65	303-316	Lys-Phe-Ile-Asp-Thr-Gln-Asn-Gly-Leu-Arg-Ile-Lys-Thr-Trp	性	-
A-66	307-320	Thr-Gln-Asn-Gly-Leu-Arg-Ile-Lys-Thr-Trp-Gln-Gly-Gly-Ser	性	-
A-67	311-324	Leu-Arg-Ile-Lys-Thr-Trp-Gln-Gly-Gly-Ser-Gly-Met-Ala-Ser	性	-
A-68	315-328	Thr-Trp-Gln-Gly-Gly-Ser-Gly-Met-Ala-Ser-His-Ile-Ile-Tyr	性	-
A-69	219-332	Gly-Ser-Gly-Met-Ala-Ser-His-Ile-Ile-Tyr-Glu-Asn-Val-Glu	性	-
A-70	223-336	Ala-Ser-His-Ile-Ile-Tyr-Glu-Asn-Val-Glu-Met-Ile-Asn-Ser	性	-
A-71	227-340	Ile-Tyr-Glu-Asn-Val-Glu-Met-Ile-Asn-Ser-Glu-Asn-Pro-Ile	性	-
A-72	231-344	Val-Glu-Met-Ile-Asn-Ser-Glu-Asn-Pro-Ile-Leu-Ile-Asn-Gln	性	-
A-73	235-348	Asn-Ser-Glu-Asn-Pro-Ile-Leu-Ile-Asn-Gln-Phe-Tyr-Cys-Thr	性	-
A-74	239-352	Pro-Ile-Leu-Ile-Asn-Gln-Phe-Tyr-Cys-Thr-Ser-Ala-Ser-Ala	性	-
A-75	243-356	Asn-Gln-Phe-Tyr-Cys-Thr-Ser-Ala-Ser-Ala-Cys-Gln-Asn-Gln	性	-
A-76	347-360	Cys-Thr-Ser-Ala-Ser-Ala-Cys-Gln-Asn-Gln-Arg-Ser-Ala-Val	性	-
A-77	351-364	Ser-Ala-Cys-Gln-Asn-Gln-Arg-Ser-Ala-Val-Gln-Ile-Gln-Asp	性	-
A-78	355-368	Asn-Gln-Arg-Ser-Ala-Val-Gln-Ile-Gln-Asp-Val-Thr-Tyr-Lys	性	-
A-79	359-372	Ala-Val-Gln-Ile-Gln-Asp-Val-Thr-Tyr-Lys-Asn-Ile-Arg-Gly	性	-
A-80	363-376	Gln-Asp-Val-Thr-Tyr-Lys-Asn-Ile-Arg-Gly-Thr-Ser-Ala-Thr	性	-
A-81	367-380	Tyr-Lys-Asn-Ile-Arg-Gly-Thr-Ser-Ala-Thr-Ala-Ala-Ile	性	-
A-82	371-384	Arg-Gly-Thr-Ser-Ala-Thr-Ala-Ala-Ile-Gln-Leu-Lys-Cys	性	-
A-83	375-388	Ala-Thr-Ala-Ala-Ile-Gln-Leu-Lys-Cys-Ser-Asp-Ser-Met	性	-
A-84	379-392	Ala-Ile-Gln-Leu-Lys-Cys-Ser-Asp-Ser-Met-Pro-Cys-Lys-Asp	性	-
A-85	383-396	Lys-Cys-Ser-Asp-Ser-Met-Pro-Cys-Lys-Asp-Ile-Lys-Leu-Ser	性	-
A-86	387-400	Ser-Met-Pro-Cys-Lys-Asp-Ile-Lys-Leu-Ser-Asp-Ile-Ser-Leu	性	-
A-87	391-404	Lys-Asp-Ile-Lys-Leu-Ser-Asp-Ile-Ser-Leu-Lys-Leu-Thr-Ser	性	-
A-88	395-408	Leu-Ser-Asp-Ile-Ser-Leu-Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala	性	-
A-89	399-412	Ser-Leu-Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn	性	-
A-90	403-416	Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn-Asp-Ala-Asn	性	-
A-91	407-420	Ile-Ala-Ser-Cys-Leu-Asn-Asp-Ala-Asn-Gly-Tyr-Phe-Ser	性	-
A-92	411-424	Leu-Asn-Asp-Ala-Asn-Gly-Tyr-Phe-Ser-Gly-His-Val-Ile	性	-
A-93	415-428	Ala-Asn-Gly-Tyr-Phe-Ser-Gly-His-Val-Ile-Pro-Ala-Cys-Lys	性	-
A-94	419-432	Phe-Ser-Gly-His-Val-Ile-Pro-Ala-Cys-Lys-Asn-Leu-Ser-Pro	性	-
A-95	423-433	Val-Ile-Pro-Ala-Cys-Lys-Asn-Leu-Ser-Pro-Ser	性	-
B-1	1-14	Asp-Asn-Pro-Ile-Asp-Ser-Cys-Trp-Arg-Gly-Asp-Ser-Asn-Trp	性	-
B-2	5-18	Asp-Ser-Cys-Trp-Arg-Gly-Asp-Ser-Asn-Trp-Ala-Gln-Asn-Arg	性	-

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
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[Table 4]

B-3	9-22	Arg-Gly-Asp-Ser-Asn-Trp-Ala-Gln-Asn-Arg-Met-Lys-Leu-Ala	性	-
B-4	13-26	Asn-Trp-Ala-Gln-Asn-Arg-Met-Lys-Leu-Ala-Asp-Cys-Ala-Val	性	-
B-5	17-30	Asn-Arg-Met-Lys-Leu-Ala-Asp-Cys-Ala-Val-Gly-Phe-Gly-Ser	性	-
B-6	21-34	Leu-Ala-Asp-Cys-Ala-Val-Gly-Phe-Gly-Ser-Ser-Thr-Met-Gly	性	-
B-7	25-38	Ala-Val-Gly-Phe-Gly-Ser-Ser-Thr-Met-Gly-Gly-Lys-Gly-Gly	性	-
B-8	29-42	Gly-Ser-Ser-Thr-Met-Gly-Gly-Lys-Gly-Gly-Asp-Leu-Tyr-Thr	性	-
B-9	33-46	Met-Gly-Gly-Lys-Gly-Gly-Asp-Leu-Tyr-Thr-Val-Thr-Asn-Ser	性	-
B-10	37-50	Gly-Gly-Asp-Leu-Tyr-Thr-Val-Thr-Asn-Ser-Asp-Asp-Pro	性	-
B-11	41-54	Tyr-Thr-Val-Thr-Asn-Ser-Asp-Asp-Pro-Val-Asn-Pro-Ala	性	-
B-12	45-58	Asn-Ser-Asp-Asp-Pro-Val-Asn-Pro-Ala-Pro-Gly-Thr-Leu	性	-
B-13	49-62	Asp-Pro-Val-Asn-Pro-Ala-Pro-Gly-Thr-Leu-Arg-Tyr-Gly-Ala	性	-
B-14	53-66	Pro-Ala-Pro-Gly-Thr-Leu-Arg-Tyr-Gly-Ala-Thr-Arg-Asp-Arg	性	-
B-15	57-70	Thr-Leu-Arg-Tyr-Gly-Ala-Thr-Arg-Asp-Arg-Pro-Leu-Trp-Ile	性	-
B-16	61-74	Gly-Ala-Thr-Arg-Asp-Arg-Pro-Leu-Trp-Ile-Ile-Phe-Ser-Gly	性	-
B-17	65-78	Asp-Arg-Pro-Leu-Trp-Ile-Ile-Phe-Ser-Gly-Asn-Met-Asn-Ile	性	-
B-18	69-82	Trp-Ile-Ile-Phe-Ser-Gly-Asn-Met-Asn-Ile-Lys-Leu-Lys-Met	性	-
B-19	73-86	Ser-Gly-Asn-Met-Asn-Ile-Lys-Leu-Lys-Met-Pro-Met-Tyr-Ile	性	-
B-20	77-90	Asn-Ile-Lys-Leu-Lys-Met-Pro-Met-Tyr-Ile-Ala-Gly-Tyr-Lys	性	-
B-21	81-94	Lys-Met-Pro-Met-Tyr-Ile-Ala-Gly-Tyr-Lys-Thr-Phe-Asp-Gly	性	-
B-22	85-98	Tyr-Ile-Ala-Gly-Tyr-Lys-Thr-Phe-Asp-Gly-Arg-Gly-Ala-Gln	性	-
B-23	89-102	Tyr-Lys-Thr-Phe-Asp-Gly-Arg-Gly-Ala-Gln-Val-Tyr-Ile-Gly	性	-
B-24	93-106	Asp-Gly-Arg-Gly-Ala-Gln-Val-Tyr-Ile-Gly-Asn-Gly-Gly-Pro	性	-
B-25	97-110	Ala-Gln-Val-Tyr-Ile-Gly-Asn-Gly-Gly-Pro-Cys-Val-Phe-Ile	性	-
B-26	101-114	Ile-Gly-Asn-Gly-Gly-Pro-Cys-Val-Phe-Ile-Lys-Arg-Val-Ser	性	-
B-27	105-118	Gly-Pro-Cys-Val-Phe-Ile-Lys-Arg-Val-Ser-Asn-Val-Ile-Ile	性	-
B-28	109-122	Phe-Ile-Lys-Arg-Val-Ser-Asn-Val-Ile-Ile-His-Gly-Leu-Tyr	性	-
B-29	113-126	Val-Ser-Asn-Val-Ile-Ile-His-Gly-Leu-Tyr-Leu-Tyr-Gly-Cys	性	-
B-30	117-130	Ile-Ile-His-Gly-Leu-Tyr-Leu-Tyr-Gly-Cys-Ser-Thr-Ser-Val	性	-
B-31	121-134	Leu-Tyr-Leu-Tyr-Gly-Cys-Ser-Thr-Ser-Val-Leu-Gly-Asn-Val	性	-
B-32	125-138	Gly-Cys-Ser-Thr-Ser-Val-Leu-Gly-Asn-Val-Leu-Ile-Asn-Glu	性	-
B-33	129-142	Ser-Val-Leu-Gly-Asn-Val-Leu-Ile-Asn-Glu-Ser-Phe-Gly-Val	性	-
B-34	133-146	Asn-Val-Leu-Ile-Asn-Glu-Ser-Phe-Gly-Val-Glu-Pro-Val-His	性	-
B-35	137-150	Asn-Glu-Ser-Phe-Gly-Val-Glu-Pro-Val-His-Pro-Gln-Asp-Gly	性	-





B-36	141-154	Gly-Val-Glu-Pro-Val-His-Pro-Gln-Asp-Gly-Asp-Ala-Leu-Thr	性	-
B-37	145-158	Val-His-Pro-Gln-Asp-Gly-Asp-Ala-Leu-Thr-Leu-Arg-Thr-Ala	陰	-
B-38	149-162	Asp-Gly-Asp-Ala-Leu-Thr-Leu-Arg-Thr-Ala-Thr-Asn-Ile-Trp	陰	-
B-39	153-166	Leu-Thr-Leu-Arg-Thr-Ala-Thr-Asn-Ile-Trp-Ile-Asp-His-Asn	陽	0.074 (0.045)
B-40	157-170	Thr-Ala-Thr-Asn-Ile-Trp-Ile-Asp-His-Asn-Ser-Phe-Ser-Asn	陰	-
B-41	161-174	Ile-Trp-Ile-Asp-His-Asn-Ser-Phe-Ser-Asn-Ser-Ser-Asp-Gly	陰	-
B-42	165-178	His-Asn-Ser-Phe-Ser-Asn-Ser-Ser-Asp-Gly-Leu-Val-Asp-Val	陰	-
B-43	169-182	Ser-Asn-Ser-Ser-Asp-Gly-Leu-Val-Asp-Val-Thr-Leu-Thr-Ser	陰	-
B-44	173-186	Asp-Gly-Leu-Val-Asp-Val-Thr-Leu-Thr-Ser-Thr-Gly-Val-Thr	陰	-
B-45	177-190	Asp-Val-Thr-Leu-Thr-Ser-Thr-Gly-Val-Thr-Ile-Ser-Asn-Asn	陰	-
B-46	181-194	Thr-Ser-Thr-Gly-Val-Thr-Ile-Ser-Asn-Asn-Leu-Phe-Asn	陰	-
B-47	185-198	Val-Thr-Ile-Ser-Asn-Asn-Leu-Phe-Asn-His-His-Lys-Val	陰	-
B-48	189-202	Asn-Asn-Leu-Phe-Asn-His-His-Lys-Val-Met-Leu-Leu-Gly	陰	-
B-49	193-206	Phe-Asn-His-Lys-Val-Met-Leu-Leu-Gly-His-Asp-Asp-Ala	陰	-
B-50	197-210	Lys-Val-Met-Leu-Leu-Gly-His-Asp-Asp-Ala-Tyr-Ser-Asp	陰	-
B-51	201-214	Leu-Gly-His-Asp-Asp-Ala-Tyr-Ser-Asp-Asp-Lys-Ser-Met-Lys	陰	-
B-52	205-218	Asp-Ala-Tyr-Ser-Asp-Asp-Lys-Ser-Met-Lys-Val-Thr-Val-Ala	陰	-
B-53	209-222	Asp-Asp-Lys-Ser-Met-Lys-Val-Thr-Val-Ala-Phe-Asn-Gln-Phe	陰	-
B-54	213-226	Met-Lys-Val-Thr-Val-Ala-Phe-Asn-Gln-Phe-Gly-Pro-Asn-Cys	陰	-
B-55	217-230	Val-Ala-Phe-Asn-Gln-Phe-Gly-Pro-Asn-Cys-Gly-Gln-Arg-Met	陰	-
B-56	221-234	Gln-Phe-Gly-Pro-Asn-Cys-Gly-Gln-Arg-Met-Pro-Arg-Ala-Arg	陰	-
B-57	225-238	Asn-Cys-Gly-Gln-Arg-Met-Pro-Arg-Ala-Arg-Tyr-Gly-Leu-Val	陰	-
B-58	229-242	Arg-Met-Pro-Arg-Ala-Arg-Tyr-Gly-Leu-Val-His-Val-Ala-Asn	陰	-
B-59	233-246	Ala-Arg-Tyr-Gly-Leu-Val-His-Val-Ala-Asn-Asn-Tyr-Asp	陰	-
B-60	237-250	Leu-Val-His-Val-Ala-Asn-Asn-Tyr-Asp-Pro-Trp-Thr-Ile	陰	-
B-61	241-254	Ala-Asn-Asn-Tyr-Asp-Pro-Trp-Thr-Ile-Tyr-Ala-Ile-Gly	陰	-
B-62	245-258	Tyr-Asp-Pro-Trp-Thr-Ile-Tyr-Ala-Ile-Gly-Gly-Ser-Ser-Asn	陰	-
B-63	249-262	Thr-Ile-Tyr-Ala-Ile-Gly-Gly-Ser-Ser-Asn-Pro-Thr-Ile-Leu	陰	-
B-64	253-266	Ile-Gly-Gly-Ser-Ser-Asn-Pro-Thr-Ile-Leu-Ser-Glu-Gly-Asn	陰	-
B-65	257-270	Ser-Asn-Pro-Thr-Ile-Leu-Ser-Glu-Gly-Asn-Ser-Phe-Thr-Ala	陰	-
B-66	261-274	Ile-Leu-Ser-Glu-Gly-Asn-Ser-Phe-Thr-Ala-Pro-Asn-Glu-Ser	陰	-
B-67	265-278	Gly-Asn-Ser-Phe-Thr-Ala-Pro-Asn-Glu-Ser-Tyr-Lys-Lys-Gln	陰	-
B-68	269-282	Thr-Ala-Pro-Asn-Glu-Ser-Tyr-Lys-Lys-Gln-Val-Thr-Ile-Arg	陰	-



B-69	273-286	Glu-Ser-Tyr-Lys-Lys-Gln-Val-Thr-Ile-Arg-Ile-Gly-Cys-Lys	性	-
B-70	277-290	Lys-Gln-Val-Thr-Ile-Arg-Ile-Gly-Cys-Lys-Thr-Ser-Ser-Ser	性	-
B-71	281-294	Ile-Arg-Ile-Gly-Cys-Lys-Thr-Ser-Ser-Ser-Ser-Ser-Ser	性	-
B-72	285-298	Cys-Lys-Thr-Ser-Ser-Ser-Ser-Ser-Ser-Ser-Ser-Ser	性	-
B-73	289-302	Ser-Ser-Cys-Ser-Ser-Ser-Ser-Ser-Ser-Ser-Ser	性	-
B-74	293-306	Asn-Trp-Val-Trp-Gln-Ser-Thr-Gln-Ser-Thr-Gln-Asp-Val	性	-
B-75	297-310	Gln-Ser-Thr-Gln-Asp-Val-Phe-Tyr-Asn-Gly-Ala-Tyr-Phe-Val	性	-
B-76	301-314	Asp-Val-Phe-Tyr-Asn-Gly-Ala-Tyr-Phe-Val-Ser-Ser-Gly-Lys	性	-
B-77	305-318	Asn-Gly-Ala-Tyr-Phe-Val-Ser-Ser-Gly-Lys-Tyr-Glu-Gly-Gly	性	-
B-78	309-322	Phe-Val-Ser-Ser-Gly-Lys-Tyr-Glu-Gly-Gly-Asn-Ile-Tyr-Thr	性	-
B-79	313-326	Gly-Lys-Tyr-Glu-Gly-Gly-Asn-Ile-Tyr-Thr-Lys-Lys-Glu-Ala	性	-
B-80	317-330	Gly-Gly-Asn-Ile-Tyr-Thr-Lys-Lys-Glu-Ala-Phe-Asn-Val-Glu	性	-
B-81	321-334	Tyr-Thr-Lys-Lys-Glu-Ala-Phe-Asn-Val-Glu-Asn-Gly-Asn-Ala	性	-
B-82	325-338	Glu-Ala-Phe-Asn-Val-Glu-Asn-Gly-Asn-Ala-Thr-Pro-Gln-Leu	性	-
B-83	329-342	Val-Glu-Asn-Gly-Asn-Ala-Thr-Pro-Gln-Leu-Thr-Lys-Asn-Ala	性	-
B-84	333-346	Asn-Ala-Thr-Pro-Gln-Leu-Thr-Lys-Asn-Ala-Gly-Val-Leu-Thr	性	-
B-85	337-350	Gln-Leu-Thr-Lys-Asn-Ala-Gly-Val-Leu-Thr-Cys-Ser-Leu-Ser	性	-
B-86	340-353	Lys-Asn-Ala-Gly-Val-Leu-Thr-Cys-Ser-Leu-Ser-Lys-Arg-Cys	性	-
C-1	74-90	Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser-Trp-Lys-Asn	性	0.084 (0.053)
C-2	91-107	Asn-Arg-Ile-Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly	性	0.089 (0.076)
C-3	192-208	Asp-Ile-Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn-Thr-Ile-Gly-Thr	性	0.071 (0.055)
C-4	352-368	Asp-Ile-Ser-Leu-Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn-Asp	性	0.081 (0.083)
700A	-	-	性	1.281 (0.051)
700B	-	-	性	0.818 (0.075)

0.077 (0.144)

註：括弧内の数値は、陰性対照におけるM492を示す。

[0030] It is shown that Table 1 thru/or the result of 6 carried out behavior from which the peptide and cedar pollen allergen with which the trial was presented differ clearly to a specific T cell to cedar pollen allergen. That is, by the system which added a sample A-19, A-20, A-23, A-48, A-49, A-89, B-39, B-80, or the cedar pollen allergen A and B, significant incorporation promotion was not accepted to incorporation promotion of clearly significant 3H-thymidine having been accepted in the system which added the sample of the complementary as compared with the negative control. This means that only the cedar pollen allergen A and B activated intentionally the T cell specific to the cedar pollen allergen in a mononuclear cell group in a sample A-19, A-20, A-23, A-48, A-49, A-89, B-39, and B-80 list.

[0031] Furthermore, the T cell activation operation carried out chemosynthesis of the peptide of the amino acid sequence shown in the array number 8 in a little low sample A-23 and the array table which consists of 17 amino acid residue separately per A-89 thru/or 11 to the sample A-19 which an amino acid sequence overlaps mutually, A-20, A-48, and A-49 list compared with other electropositive samples.

[0032] That is, milli JIEN / peptide synthesis machine made from a biotechnology research "Excel" was used, the peptide (a sample C-1 thru/or C-4) of the amino acid sequence shown in the array number 8 in an array table thru/or 11 was separately compounded according to the conventional method, and the reversed phase high pressure liquid chromatography which uses the Biorad chromatography column "Hi-Pore RP-318 mold" refined to 95% of purity, respectively. When a sample C-1 thru/or a part of C-4 were taken after purification and the Perkin-Elmer peptide sequencer "470A molds" analyzed, four kinds of all peptides concerning composition had the expected amino acid sequence.

[0033] When examined like the above these samples C-1 thru/or per C-4, all are positivities and it became clear that a specific T cell was intentionally activated to cedar pollen allergen.

[0034]

[Reactivity over an immunoglobulin E antibody specific to example of experiment 2-2 cedar pollen allergen] A specific T cell in the sample B-39 from which it became clear to be intentionally activated to cedar pollen allergen in the example 2-1 of an experiment, B-80, C-1, or C-4 list to Allergen A and B EIA which TANIAI and others has reported to "molecular immunology", the 30th volume, No. 2, and the 183-189th page (1993) -- law was applied and reactivity with a specific immunoglobulin E antibody was

investigated to the cedar pollen allergen extracted from a hay fever patient's blood.

[0035] namely, the product made from a pierced earring -- cross linking agent "(sulfo succinimidyl) SUBERATO (BS3)" 1g was dissolved in 10ml of distilled water, and it poured distributively 50microl / well every to the Nunc "KOBARINKU mold" 96 well microplate, and incubated at 37 degrees C for 3 hours. Distilled water washes a microplate, the sample B-39 prepared in the examples 1 and 2 of an experiment, B-80, C-1, C-4, or Allergen A and B is dissolved in PBS so that it may become [ ml ] in 20microg [ ml ] /or 5microg /, 50microl / well distributive pouring is carried out at a microplate, it incubated at 37 degrees C for further 3 hours, and covalent bond was carried out to the microplate. and PBS which contains bovine serum albumin 0.1% (w/v) after putting PBS which contains bovine serum albumin in a microplate 1% (w/v) at 50microl / well \*\*\*\*, and 4 degrees C overnight and blocking an unreacted active group -- washing -- bovine serum albumin -- tales-doses \*\*\*\* -- a hay fever patient's blood serum diluted with fresh PBS 5 times was made to react at 50microl / well \*\*\*\*, and 37 degrees C for 1 hour

[0036] Next, a microplate is washed by PBS which contains bovine serum albumin 0.1% (w/v). KIRUKE guard - and the - Perry biotin indicator anti-Homo sapiens epsilon chain antibody which were diluted [ ml ] with fresh PBS in 1microg /are added 50microl / well every. bovine serum albumin -- tales-doses \*\*\*\* -- After incubating at 37 degrees C for 1 hour, again by PBS which contains bovine serum albumin 0.1% (w/v) After washing, bovine serum albumin -- tales-doses \*\*\*\* -- the peroxidase labelling avidin made from ZAIMEDDO diluted with fresh PBS 5,000 times was incubated at 50microl / well \*\*\*\*, and 37 degrees C for further 1 hour. And after washing, under 100microl / well \*\*\*\*, and a room temperature, the 0.1M citric-acid-phosphate buffer (pH5.0) which contains 0.03% (v/v) and 0.5mg /of alt.phenylenediamines for a hydrogen peroxide ml was put for 5 minutes, and carried out the enzyme reaction by PBS which contains bovine serum albumin for a microplate 0.1% (w/v). After adding 2-N sulfuric acid 100microl / well every and stopping a reaction, the absorbance under the wavelength of 492nm was measured by the well-known approach of using a spectrophotometer.

[0037] The system which replaces with a cedar pollen patient's blood serum, and uses a healthy person's blood serum for coincidence was prepared, a measure was taken similarly, and it considered as the negative control. A result is shown in Table 1 thru/or 6.

[0038] A sample B-39, B-80 and C-1 thru/or C-4 did not react substantially to Allergen A and B having reacted to the cedar pollen allergen of the hay fever patient origin to a specific immunoglobulin E antibody strongly so that clearly from the result shown in Table 1 thru/or 6. This means lacking the B cell epitope of the cedar pollen allergen by which these samples are contained

in Allergen A and B. If these results and the result of the example 2-1 of an experiment are judged synthetically, it will be judged that the peptide of the amino acid sequence shown in the above-mentioned sample 8, i.e., the array number in an array table, thru/or 13 is what essentially consists of a T cell epitope of cedar pollen allergen.

[0039]

[Retrieval of an amino acid sequence indispensable in order that an example of experiment 3 T cell may recognize a T cell epitope] Six kinds of T cell epitopes clarified in the example 2 of an experiment were analyzed further, and in order that a T cell might recognize them, the indispensable amino acid sequence was searched with this example of an experiment.

[0040] That is, lessons was taken from the amino acid sequence of the sample B-38 which denied the positivity in the amino acid sequence list shown in the array number 8 in an array table thru/or 13, and did not go out in Table 5 thru/or 6 in it, B-81, and B-82 by the approach of the example 1-1 of an experiment, and chemosynthesis of the various peptides which consist of 14 amino acid which permuted 1 of the amino acid of those ends or both ends or two pieces or more by the alanine, or 17 pieces was carried out. And the reactivity over an immunoglobulin E antibody specific to activation and cedar pollen allergen of a T cell specific to cedar pollen allergen was investigated by the approach of the example 2 of an experiment about these peptides.

[0041] Consequently, as shown in Table 7, it became clear that the sample D-1 which comes to contain the amino acid sequence shown in the array number 1 of an array table thru/or 7 thru/or the peptide of D-7 showed the almost same behavior as the peptide of the amino acid sequence shown in the array number 8 thru/or 13 to an immunoglobulin E antibody specific to a T cell specific to cedar pollen allergen and cedar pollen allergen. The amino acid sequence which this shows to the array number 1 of an array table thru/or 7 has suggested strongly that it is an indispensable array, in order that a T cell may recognize the peptide of the amino acid sequence shown in the array number 8 thru/or 13.

[0042]

[Table 7]

試料	アミノ酸配列	T細胞の活性化	ヒトイムノグロブリンE抗体 に対する反応性 (A492)
D-1	Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Ala-Ala-Ala-Ala-Ala-Ala	陽性	0.079 (0.092)
D-2	Ala-Ala-Ala-Ala-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser-Ala-Ala-Ala	陽性	0.082 (0.063)
D-3	Asn-Arg-Ile-Trp-Leu-Gln-Phe-Ala-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Ala	陽性	0.091 (0.059)
D-4	Ala-Ala-Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Ala-Ala-Ala-Ala	陽性	0.085 (0.071)
D-5	Ala-Ala-Ser-Leu-Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Ala-Ala-Ala	陽性	0.088 (0.089)
D-6	Leu-Thr-Leu-Arg-Thr-Ala-Thr-Asn-Ala-Ala-Ala-Ala-Phe-Asn-Val-Glu	陽性	0.076 (0.069)
D-7	Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Phe-Asn-Val-Glu	陽性	0.089 (0.076)

[0043] As explained above, this invention does not react to an immunoglobulin E antibody specific to cedar pollen allergen substantially, but when it examines by the approach of judging by the incorporation of 3H-thymidine, it relates to the peptide which activates a specific T cell intentionally to cedar pollen allergen as compared with a negative control. As long as a peptide possesses this property, with respect to that structure, a source and the origin, and the preparation approach, this invention shall not exist and shall be included altogether.

[0044] the peptide of this invention -- usually -- 5 -- or 10 thru/or 20 amino acid come to carry out peptide linkage of the 50 pieces desirably As each peptide, what has the amino acid sequence shown in the array number 8 in an array table thru/or 13, and the thing which has a homonous amino acid sequence in these amino array are mentioned, for example. Without changing the above-mentioned immunological operation substantially, the peptide of a homonous amino acid sequence can permute two or more pieces from 1 of the amino acid in the amino acid sequence shown in the array number 8 in an array table thru/or 13, or other amino acid, or can obtain proper amino acid one piece or by combining two or more pieces to the end or both ends of these amino acid sequences.

[0045] In order that a T cell may recognize them in the amino acid sequence shown in the array number 8 of an array table thru/or 13, only an indispensable amino acid sequence is made eternal and, specifically, other amino acid permutes the other amino acid in the range which does not change substantially the immunological operation as a T cell epitope of cedar pollen. Or one piece or the peptide which is combined two or more pieces and obtained makes amino acid the number of amino acid residue, usual [ the die length which can be recognized, i.e., usual, /, such as an alanine, / of a T cell ], as a whole, and it is made to become 10 thru/or 20 pieces suitably if needed to the indispensable end or indispensable both ends of an amino acid sequence. As this amino acid sequence, the peptide of the amino acid sequence which the amino acid sequence shown in the array number 1 in an array table thru/or 7 is mentioned, and is shown in the array number 16 in an array table thru/or 24 as an example of this homologue can be mentioned, for example.

[0046] The peptide of this invention can be easily prepared with the peptide synthesis method of common use in the field known as a "solid phase technique" or a "liquid phase process." Although detailed explanation is omitted since this invention does not relate to the peptide synthesis itself, the detail of peptide synthesis is indicated by the Tokyo Kagaku Dojin issue in the edited by Japanese Biochemical Society "a new chemistry experiment lecture", the 1st volume, "protein VI", the 3-44th page, and 1992, for example. However, the peptide of this invention may not be limited to what was prepared



by chemosynthesis, for example, may decompose suitably the cedar pollen allergen which extracted from the pollen or the male of a Japan cedar, or was prepared by recombinant DNA technology, and may extract it from a decomposition product. Or DNA which carries out the code of the peptide which has the amino acid sequence shown in the array number 8 in an array table thru/or 13 or an amino acid sequence homonous to them, for example is prepared, it inserts in the vector which can replicate this autonomously and considers as a recombinant DNA, and Escherichia coli, a Bacillus subtilis, an Actinomyces, yeast, etc. may introduce this into a host suitably, it may consider as a transformant, and the peptide of this invention may be extracted from that culture. DNA which carries out the code of the peptide of the amino acid sequence shown in the array number 8 in an array table thru/or 13 can be prepared based on the base sequence of cDNA indicated by for example, the Japanese-Patent-Application-No. No. 344596 [ five to ] specification, and the international patent public presentation/[ 93rd ] No. 01213 specification. Furthermore, the peptide of this invention may be a gestalt as the gestalt, the derivative which is made to carry out the bridge formation polymerization of the peptide with acetylation, amidation, and/or a polyfunctional reagent, and is obtained further, or polymer as complex which adds sugar and a polyethylene glycol to the peptide obtained thus, and is obtained.

[0047] The peptide of this invention is usually refined in advance of use, although expected therapy and preventive effect are demonstrated even if it prescribes a medicine for the patient with a comparatively \*\*\*\* gestalt. What is necessary is to use the approach of the common use in the field for refining a peptide thru/or protein, such as filtration, concentration, centrifugal separation, gel filtration chromatography, an ion-exchange chromatography GUFURA fee, a high speed liquid chromatography, affinity chromatography, gel electrophoresis, and isoelectric focusing, for purification, and just to combine these approaches with it suitably if needed. And what is necessary is to condense and freeze-dry the refined peptide according to an end-use gestalt, and just to make it liquefied or a solid state.

[0048] Since the peptide of this invention does not react to an immunoglobulin E antibody specific to cedar pollen allergen substantially but a specific T cell is moreover intentionally activated to cedar pollen allergen as above-mentioned, it has an application extensive as an immunotherapy agent for treating and preventing hay fever. The immunotherapy agent which comes to contain the peptide of this invention as an active principle can treat hay fever, without causing side effects, such as anaphylaxis, substantially, if the general mammals including the Homo sapiens suffered from hay fever are medicated. When medicating a healthy individual and the individual of potential hay fever with the immunotherapy agent of this invention before cedar pollen begins to disperse, while demonstrating a remarkable preventive effect to hay fever on the other

hand, higher efficacy is demonstrated to the remission of the allergy symptom at the time of the onset.

[0049] one sort of the peptide usually according [ the immunotherapy agent of this invention ] to this invention if it explains to the immunotherapy agent per pan of this invention in detail, or two sorts or more -- 0.01 thru/or 100% (w/w) -- desirable -- 0.05 thru/or 50% (w/w) -- further -- desirable -- 0.5 -- or it comes to contain 5.0% (w/w) The immunotherapy agent of this invention includes further the gestalt as a constituent with support, such as the serum albumin from the first with another gestalt peptide independent [ concerned ] permitted physiologically, gelatin, a mannitol, a maltose, and trehalose, an excipient, an immunoadjuvant, a stabilizer, one sort containing anti-inflammatory agents and antihistamines, such as steroid hormone and chestnut MOGURIKU acid sodium, or two sorts or more of other drugs if needed. Furthermore, the immunotherapy agent of this invention also includes the drugs of medication unit form voice, and the drugs of that medication unit form voice contain the amount which is equivalent to the dosage per day, its integral multiple (up to 4 times), or its divisor in the polypeptide of this invention (to 1/40), and mean the drugs in the pharmaceutical form of one suitable for administration separated physically. As drugs of such medication unit form voice, powder, a fine grain agent, a granule, a pill, a tablet, a capsule, the trochiscus, syrups, an emulsion, an ointment, plaster, cataplasms, suppositories, ophthalmic solutions, a nasal drop, a spray, injections, etc. are mentioned.

[0050] the general mammals in which the immunotherapy agent of this invention contains Homo sapiens for the purpose of the therapy and prevention of hay fever when the operation of the immunotherapy agent of this invention is explained -- transderma, taking orally, and the rhinenchysis -- a medicine is applied eyewash or injection prescribed for the patient although the dose in Homo sapiens changes even if it depends on the purpose and symptom of administration, while usually observing a candidate's symptom and the progress after administration -- an adult -- per [ 0.01 ] day thru/or 1.0g -- desirable -- 0.01 thru/or 0.1g -- a standard -- 1 time of 1 time of every week thru/or every month of frequency -- it is -- about 1 -- or repeated-dose administration is usually carried out for six months, increasing a dosage.

[0051] Hereafter, the example of 2-3 is given and explained about preparation of a peptide and the application by this invention.

[0052]

[Preparation of an example A-1 peptide] Milli JIEN / peptide synthesis machine made from a biotechnology research "Excel" was used, the peptide of the amino acid sequence shown in the array number 8 in an array table thru/or 11 was separately compounded according to the conventional method, and it

freeze-dried after purification to 95% of purity, respectively with the reversed phase high pressure liquid chromatography which uses the Biorad chromatography column "Hi-Pore RP-318 mold", and considered as the solid state material. When a part of solid state material was taken and the Perkin-Elmer peptide sequencer "470A molds" analyzed, four kinds of all peptides concerning composition had the expected amino acid sequence.

[0053]

[Preparation of an example A-2 peptide] The peptide synthesis kit made from the Cambridge research biochemicals "multipin" was used, and chemosynthesis of the peptide of the amino acid sequence shown in the array numbers 12 and 13 in an array table was separately carried out according to the conventional method, and like the example A-1, it freeze-dried after purification to 95% of purity, and considered as the solid state material, respectively. When a part of solid state material was taken and it was similarly analyzed as the example A-1, all had the expected amino acid sequence.

[0054]

[Preparation of an example A-3 peptide] Like the example A-1, chemosynthesis of the peptide of the amino acid sequence shown in the array number 16 in an array table was carried out, and it was refined to 95% of purity. When some peptides were taken after purification and it was similarly analyzed as the example A-1, it had the expected amino acid sequence.

[0055]

[Preparation of an example A-4 peptide] Like the example A-2, chemosynthesis of the peptide of the amino acid sequence shown in the array number 17 in an array table was carried out, and it was refined to 95% of purity. When some peptides were taken after purification and it was similarly analyzed as the example A-1, it had the expected amino acid sequence.

[0056]

[Preparation of an example A-5 peptide] Chemosynthesis of the peptide of the amino acid sequence shown in the array number 18 in the array table equivalent to the sample D-1 of the example 3 of an experiment thru/or D-7 thru/or 24 like an example A-1 thru/or A-2 was carried out, and it freeze-dried after purification to 95% of purity, and considered as the solid state material, respectively. When a part of solid state material was taken and the Perkin-Elmer peptide sequencer "470 molds" analyzed, seven kinds of all peptides concerning composition had the expected amino acid sequence.

[0057]

[Example B-1 liquids and solutions] It dissolved in distilled water which contains purified gelatin 1% (w/v) as a stabilizer so that it might become the last concentration of 0.1g/ml about either of six kinds of peptides obtained by the example A-1 and the approach of A-2, and sterilization filtration was carried out with the conventional method, and six kinds of liquids and solutions

were obtained.

[0058] Since usually changes for every individual as for the susceptibility over the peptide of this invention, this article uses six kinds of liquids and solutions, blending them suitably so that it may become the presentation which was most suitable for each individual. This article excellent in stability is useful as liquids and solutions for the ophthalmic solutions for treating and preventing hay fever, a nasal drop, and the sprays in the oral cavity.

[0059]

[Example B-2 injections] six kinds of peptides obtained by the example A-1 and the approach of A-2 to the physiological saline which contains a human serum albumin 1% (w/v) as a stabilizer -- respectively -- last concentration 0. -- after dissolving and carrying out sterilization filtration so that it may become [ ml ] in 0.1, 0.1, or 1mg /, 2ml was poured distributively into each sterilization vial bottle, and it freeze-dried and sealed into it.

[0060] In advance of administration, first, this article adds 1ml of distilled water for injection etc. in a vial bottle, and, subsequently to homogeneity, dissolves and uses contents. This article which is excellent in stability and comes to contain six kinds of polypeptides by this invention as an active principle is useful as desiccation injections for treating and preventing hay fever.

[0061]

[Example B-3 tablet] It was made to react at 5 degrees C under churning for 2 hours, dissolving with an average molecular weight of about 20,000dalton purification pullulan 2g in 100ml of distilled water at homogeneity, adding 2ml of 1.7% (w/v) acetone solutions of cyanuric chloride to a solution, and a sodium-carbonate water solution maintaining pH at the seven neighborhoods 5% (w/v). Then, keeping pH of a reactant the same to the seven neighborhoods, it dialyzed to 4-degree C cold water overnight, and 20ml of water solutions containing an activation pullulan was obtained.

[0062] The peptide of the amino acid sequence shown in the array numbers 8, 10, and 11 in the array table obtained by the approach of an example A-1 in this water solution, The peptide of the amino acid sequence shown in the array table obtained by the approach of an example A-2 at the array number 13, It was made to react at 37 degrees C for 12 hours, agitating gently adding the peptide obtained by the approach of an example A-3, and 0.2mg of peptides obtained by the approach of an example A-4, respectively, and maintaining pH of a solution at the seven neighborhoods. Having added 4g of glycines to the reactant after the reaction, and agitating gently, it incubated at 37 degrees C for 5 hours, and the unreacted active group was blocked.

[0063] The reactant was condensed, the load was carried out to G-sephadex 50 column made to equilibrate with 0.1M phosphate buffer (pH7.0) beforehand, the same buffer solution fresh to a column was dipped, and the fraction containing the peptide of this invention and the complex of a pullulan was

extracted. Yield was about 30% per raw material peptide solid content.

[0064] According to the conventional method, sterilization filtration was carried out, this fraction was condensed, it freeze-dried, the mannitol was mixed to homogeneity after grinding, mixture was tableted, and 2, 10, or the tablet included 50mg was obtained for product 1 lock (200mg) per complex.

[0065] This article excellent in intake nature and stability is useful as a hypoglottis agent for treating and preventing hay fever.

[0066]

[Example B-4 syrups] Dissolved 1g of purification lipopolysaccharide of the Escherichia coli origin in 100ml of 10mM calcium phosphate water solutions, added 6ml of 100mM periodic acid sodium to the solution, it was made to react for 20 minutes under a room temperature, and lipopolysaccharide was activated. After dialyzing a reactant overnight to the 4-degree C 1M glycine-hydrochloric-acid buffer solution (pH4.4) and removing unreacted periodic acid, While the 0.1M sodium-hydrogencarbonate buffer solution adjusts to the pH9.5 neighborhood It dissolves at a time separately 10mg of six kinds of peptides obtained by the example A-1 and the approach of A-2 in 100ml (pH7.0) of 0.1M phosphate buffers, respectively, and it put for 12 hours and was made to react under a room temperature in addition to the above-mentioned reactant containing activation lipopolysaccharide.

[0067] Then, the fraction which refines the newly obtained reactant by the approach of an example B-3, and contains the peptide of this invention and the complex of lipopolysaccharide which were obtained was condensed, and it freeze-dried, and it ground and considered as the solid state material. Yield was about 30% per raw material peptide solid content.

[0068] It dissolved in distilled water which contains purified gelatin 1% (w/v) as a stabilizer so that the last concentration might become 0.1, 1mg [ ml ] /, or 50% (w/w) about this solid state material and sucrose, respectively, and sterilization filtration of the solution was carried out with the conventional method, and the sirupy object was obtained. It poured distributively and sealed 2ml of this sirupy object at a time into the sterilization vial bottle, and considered as the product.

[0069] This article which is excellent in stability and contains the peptide of this invention and the complex of lipopolysaccharide as an active principle is useful as syrups for treating and preventing hay fever.

[0070]

[Example B-5 liquids and solutions] It dissolved in distilled water which contains purified gelatin 1% (w/v) as a stabilizer so that it might become the last concentration of 0.1g/ml about either of seven kinds of peptides obtained by the approach of an example A-5, and sterilization filtration was carried out with the conventional method, and seven kinds of liquids and solutions were obtained.

[0071] Since usually changes for every individual as for the susceptibility over the peptide of this invention, this article uses seven kinds of liquids and solutions, blending them suitably so that it may become the presentation which was most suitable for each individual. This article excellent in stability is useful as liquids and solutions for the ophthalmic solutions for treating and preventing hay fever, a nasal drop, and the sprays in the oral cavity.

[0072]

[Example B-6 injections] seven kinds of peptides obtained by the approach of an example A-5 to the physiological saline which contains a human serum albumin 1% (w/v) as a stabilizer -- respectively -- last concentration 0. -- after dissolving and carrying out sterilization filtration so that it may become [ ml ] in 0.1, 0.1, or 1mg /, 2ml was poured distributively into each sterilization vial bottle, and it freeze-dried and sealed into it.

[0073] In advance of administration, first, this article adds 1ml of distilled water for injection etc. in a vial bottle, and, subsequently to homogeneity, dissolves and uses contents. This article which is excellent in stability and comes to contain seven kinds of polypeptides by this invention as an active principle is useful as desiccation injections for treating and preventing hay fever.

[0074]

[Example B-7 syrups] Seven kinds of peptides which obtained purified gelatin by the approach of an example A-5 to distilled water included 1% (w/v) were dissolved, respectively so that it might become 50% (w/v) about ml, 0.1mg /, and sucrose, sterilization filtration of the solution was carried out with the conventional method, and the sirupy object was obtained. It poured distributively and sealed 2ml of this sirupy object at a time into the sterilization vial bottle, and considered as the product.

[0075] This article which is excellent in stability and contains the peptide of this invention as an active principle is useful as syrups for treating and preventing hay fever.

[0076]

[Example of experiment 4 acute toxicity test] the immunotherapy agent obtained by the example B-1 thru/or the approach of B-7 to the day [ of after the birth / 20th ] mouse the conventional method -- taking orally -- or it injected intraperitoneally. Consequently, as for these immunotherapy agent, it became clear that it was fifty percent lethal dose of 200 or more mg/kg according to any route of administration. This shows that combination use can be carried out to insurance at the immunotherapy agent with which the mammals in which the peptide of this invention contains Homo sapiens are medicated.

[0077]

[Effect of the Invention] As explained above, this invention is based on discovery of the peptide which essentially consists of a T cell epitope of cedar

pollen allergen. The peptide of this invention activates a specific T cell to cedar pollen allergen, without causing anaphylaxis substantially, if the mammals including Homo sapiens are medicated since it does not react to an immunoglobulin E antibody specific to cedar pollen allergen substantially. therefore -- if the mammals including Homo sapiens are medicated with the immunotherapy agent of this invention that comes to contain this peptide as an active principle -- a side effect -- it is few and remarkable therapy and preventive effect are demonstrated to hay fever for a short period of time. And the peptide of this invention can manufacture the amount of requests easily, and since quality control is also easy, it can use it for the therapy and prevention of hay fever extremely at insurance.

[0078] This invention to which \*\*\*\* also demonstrates the remarkable operation effectiveness can be called invention which has a great meaning in contributing to the field sincerity.

[0079]

[Layout Table]

array number: -- die-length [ of one array ]: -- mold [ of 11 arrays ]: -- amino acid topology: -- class [ of straight chain-like array ]: -- peptide array Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn1 5 10 [0080] array number: -- die-length [ of two arrays ]: -- mold [ of ten arrays ]: -- amino acid topology: -- class [ of straight chain-like array ]: -- peptide array Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser1 5 10 [0081] array number: -- die-length [ of three arrays ]: -- mold [ of ten arrays ]: -- amino acid topology: -- class [ of straight chain-like array ]: -- peptide array Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu1 5 10 [0082] array number: -- die-length [ of four arrays ]: -- mold [ of ten arrays ]: -- amino acid topology: -- class [ of straight chain-like array ]: -- peptide array Phe Ala Ser Lys Asn Phe His Leu Gln Lys1 5 10 [0083] array number: -- die-length [ of five arrays ]: -- mold [ of 11 arrays ]: -- amino acid topology: -- class [ of straight chain-like array ]: -- peptide array Ser Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser1 5 10 [0084] array number: -- die-length [ of six arrays ]: -- mold [ of eight arrays ]: -- amino acid topology: -- class [ of straight chain-like array ]: -- peptide array Leu Thr Leu Arg Thr Ala Thr Asn1 5 [0085] array number: -- die-length [ of seven arrays ]: -- mold [ of five arrays ]: -- amino acid topology: -- class [ of straight chain-like array ]: -- peptide array Ala Phe Asn Val Glu1 5 [0086] array number: -- die-length [ of eight arrays ]: -- mold [ of 17 arrays ]: -- amino acid topology: -- class [ of straight chain-like array ]: -- peptide array Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser Trp Lys Asn 1 5 10 15 [0087] array number: -- die-length [ of nine arrays ]: -- mold [ of 17 arrays ]: -- amino acid topology: -- class [ of straight chain-like array ]: -- peptide array Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu Met Gly 1 5 10 15 [0088] array number: -- die-length [ of ten arrays ]: -- mold [ of 17 arrays ]: -- amino acid topology: -- class [ of straight chain-like array





Cys Lys 375 380 385 390 Asp Ile Lys Leu Ser Asp Ile Ser Leu Lys Leu Thr Ser  
 Gly Lys Ile Ala 395 400 405 Ser Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe  
 Ser Gly His Val Ile Pro 410 415 420 425 AlaCysLys Asn Leu SerPro Ser Ala  
 Lys Arg Lys Glu Ser Lys Ser His 430 435 440 Lys His Pro Lys Thr Val Met Val  
 Lys Asn Met Gly Ala Tyr Asp Lys Gly 445 450 455 Asn Arg Thr Arg Ile Leu  
 Leu Gly Ser Arg Pro Pro Asn Cys Thr Asn Lys 460 465 470 475 Cys His Gly  
 Cys Ser Pro Cys Lys Ala Lys Leu Val Ile Val His Arg Ile 480 485 \*\*\*\*\* .  
 \*\*\*\*\*. \*\*\*\*\*. \*\*\*\*\*. \*\*\*\*\*(1). \*\*\*\*\*. \*\*\*\*\*. \*\*\*\*\*. \*\*\*\*\*. \*\*\*\*\*.  
 \*\*\*\*\*. \*\*\*\*\*. \*\*\*\*\*. \*\*\*\*\*. \*\*\*\*\*. \*\*\*\*\*. \*\*\*\*\*. \*\*\*\*\*. \*\*\*\*\*. \*\*\*\*  
 machine . \*\*\*\*\* . \*\*\*\* machine . \*\*\*\*\* . \*\*\*\*\*. \*\*\*\*\*. \*\*\*\*\*. \*\*\*\*\*  
 [0093] array number: -- die-length [ of 15 arrays ]: -- mold [ of 353 arrays ]:  
 -- amino acid topology: -- class [ of straight chain-like array ]: -- protein  
 origin living thing name: -- a *Cryptomeria japonica* individual and isolation living  
 thing name: -- Japan cedar array Asp Asn Pro Ile Asp Ser Cys Trp Arg Gly  
 Asp Ser Asn Trp Ala Gln Asn 1 5 10 15 Arg Met Lys Leu Ala Asp Cys Ala Val  
 Gly Phe Gly Ser Ser Thr Met Gly 20 25 30 Gly Lys Gly Gly AspLeu Tyr Thr Val  
 Thr Asn Ser Asp Asp Asp Pro Val 35 40 45 50 Asn Pro Ala Pro Gly Thr Leu Arg  
 Tyr Gly Ala Thr Arg Asp Arg Pro Leu 55 60 65 Trp Ile Ile Phe Ser Gly Asn Met  
 Asn Ile Lys Leu Lys Met Pro Met Tyr 70 75 80 85 Ile Ala Gly Tyr Lys Thr Phe  
 Asp Gly Arg Gly Ala Gln Val Tyr Ile Gly 90 95 100 Asn Gly Gly Pro Cys Val  
 Phe Ile Lys Arg Val Ser Asn Val Ile Ile His 105 110 115 Gly Leu Tyr Leu Tyr  
 Gly Cys Ser Thr Ser Val Leu Gly Asn Val Leu Ile 120 125 130 135 AsnGlu Ser  
 Phe Gly Val Glu Pro Val His Pro Gln Asp Gly Asp Ala Leu 140 145 150 Thr Leu  
 Arg Thr Ala Thr Asn Ile Trp Ile Asp His Asn Ser Phe Ser Asn 155 160 165 170  
 Ser Ser Asp Gly Leu ValAsp Val Thr Leu Thr Ser Thr Gly Val Thr Ile 175 180  
 185 Ser Asn Asn Leu Phe Phe Asn His His Lys Val Met Leu Leu Gly His Asp  
 190 195 200 Asp Ala Tyr Ser Asp Asp Lys Ser Met Lys Val Thr Val Ala Phe  
 Asn Gln 205 210 215 220 Phe GlyProAsn Cys GlyGln Arg Met Pro Arg Ala Arg  
 Tyr Gly Leu Val 225 230 235 His Val Ala Asn Asn Asn Tyr Asp Pro Trp Thr Ile  
 Tyr Ala Ile Gly Gly 240 245 250 255 Ser Ser Asn ProThr Ile Leu SerGlu Gly Asn  
 Ser Phe Thr Ala Pro Asn 260 265 270 Glu Ser Tyr LysLys GlnVal Thr Ile Arg  
 Ile Gly Cys Lys Thr Ser Ser 275 280 285 Ser CysSerAsn Trp Val Trp Gln Ser  
 Thr Gln Asp Val Phe Tyr Asn Gly 290 295 300 305 Ala Tyr PheValSer Ser  
 GlyLys Tyr Glu Gly Gly Asn Ile Tyr Thr Lys 310 315 320 Lys Glu Ala Phe Asn  
 Val Glu Asn Gly Asn Ala Thr Pro Gln Leu Thr Lys 325 330 335 340 Asn Ala Gly  
 Val Leu Thr Cys Ser Leu Ser Lys Arg Cys 345 350 [0094] array number: --  
 die-length [ of 16 arrays ]: -- mold [ of 17 arrays ]: -- amino acid topology: --  
 class [ of straight chain-like array ]: -- peptide array Asn Arg Ile Trp Leu Gln  
 Phe Ala Lys Leu Gln Gly Phe Thr Leu Met Gly 1 5 10 15 [0095] array number:  
 -- die-length [ of 17 arrays ]: -- mold [ of 14 arrays ]: -- amino acid topology:  
 -- class [ of straight chain-like array ]: -- peptide array Leu Ala Leu Arg Thr  
 Ala Thr Asn Ile Trp Ile Asp His Asn 1 5 10 [0096] array number: -- die-length [

of 18 arrays ]: -- mold [ of 17 arrays ]: -- amino acid topology: -- class [ of  
straight chain-like array ]: -- peptide array Lys Val Asp Gly Ile Ile Ala Ala Tyr  
Gln Asn Ala Ala Ala Ala Ala Ala 1 5 10 15 [0097] array number: -- die-length [  
of 19 arrays ]: -- mold [ of 17 arrays ]: -- amino acid topology: -- class [ of  
straight chain-like array ]: -- peptide array Ala Ala Ala Ala Ile Ile Ala Ala Tyr  
Gln Asn Pro Ala Ser Ala Ala Ala 1 5 10 15 [0098] array number: -- die-length [  
of 20 arrays ]: -- mold [ of 17 arrays ]: -- amino acid topology: -- class [ of  
straight chain-like array ]: -- peptide array Asn Arg Ile Trp Leu Gln Phe Ala  
Lys Leu Ala Ala Ala Ala Ala Ala 1 5 10 15 [0099] array number: --  
die-length [ of 21 arrays ]: -- mold [ of 17 arrays ]: -- amino acid topology: --  
class [ of straight chain-like array ]: -- peptide array Ala Ala Phe Ala Ser Lys  
Asn Phe His Leu Gln Lys Ala Ala Ala Ala 1 5 10 15 [0100] array number: --  
die-length [ of 22 arrays ]: -- mold [ of 17 arrays ]: -- amino acid topology: --  
class [ of straight chain-like array ]: -- peptide array Ala Ala Ser Leu Lys Leu  
Thr Ser Gly Lys Ile Ala Ser Ala Ala Ala Ala 1 5 10 15 [0101] array number: --  
die-length [ of 23 arrays ]: -- mold [ of 14 arrays ]: -- amino acid topology: --  
class [ of straight chain-like array ]: -- peptide array Leu Thr Leu Arg Thr Ala  
Thr Asn Ala Ala Ala Ala Ala Ala 1 5 10 [0102] array number: -- die-length [ of  
24 arrays ]: -- mold [ of 14 arrays ]: -- amino acid topology: -- class [ of  
straight chain-like array ]: -- peptide array Ala Ala Ala Ala Ala Ala Ala Ala  
Ala Phe Asn Val Glu 1 5 10

[Translation done.]

**\* NOTICES \***

JP 8-127591

**JPO and NCIP are not responsible for any damages caused by the use of this translation.**

1. This document has been translated by computer. So the translation may not reflect the original precisely.
2. \*\*\*\* shows the word which can not be translated.
3. In the drawings, any words are not translated.

**CLAIMS**

[Claim(s)]

[Claim 1] The peptide which will activate a specific T cell intentionally to cedar pollen allergen as compared with a negative control if it examines by the approach which does not react to an immunoglobulin E antibody specific to cedar pollen allergen substantially, but is judged by the incorporation of 3H-thymidine.

[Claim 2] The peptide according to claim 1 which comes to contain either of the amino acid sequences shown in the array number 1 in an array table thru/or 7.

[Claim 3] The peptide according to claim 1 or 2 which comes to contain one which is shown in the array number 8 in an array table thru/or 13 of amino acid sequences, or an amino acid sequence homonous to the amino acid sequence.

[Claim 4] An amino acid sequence given in the array number 1 of the array table in a peptide according to claim 1.

[Claim 5] An amino acid sequence given in the array number 2 of the array table in a peptide according to claim 1.

[Claim 6] An amino acid sequence given in the array number 3 of the array table in a peptide according to claim 1.

[Claim 7] An amino acid sequence given in the array number 4 of the array table in a peptide according to claim 1.

[Claim 8] An amino acid sequence given in the array number 5 of the array table in a peptide according to claim 1.

[Claim 9] An amino acid sequence given in the array number 6 of the array table in a peptide according to claim 1.

[Claim 10] An amino acid sequence given in the array number 7 of the array table in a peptide according to claim 1.

[Claim 11] The immunotherapy agent which comes to contain a peptide according to claim 1 to 3 as an active principle.

[Claim 12] They are 0.01 thru/or the immunotherapy agent according to claim 11 which it comes to contain 100% (w/w) about a peptide according to claim 1 to 3 as an active principle.

[Claim 13] The immunotherapy agent according to claim 11 or 12 which contains serum albumin, gelatin, a mannitol, a maltose, and/or trehalose as a stabilizer or an excipient.

[Translation done.]